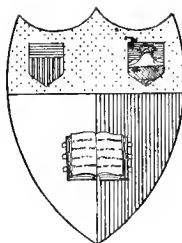


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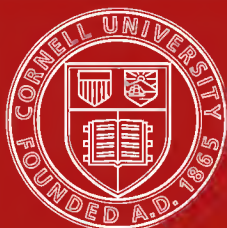
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J. V. L. Foreman.

THE DETERMINATION OF HYDROGEN IONS

An elementary treatise on the hydrogen electrode, indicator and supplementary methods with an indexed bibliography on applications

BY

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To
Fellow Workers in the Biological Sciences,
Architects of Progress,
Who Hew the Stone to Build Where Unseen Spires Shall Stand

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PREFACE

Poincaré in *The Foundations of Science* remarks, "There are facts common to several sciences, which seem the common source of streams diverging in all directions and which are comparable to that knoll of Saint Gothard whence spring waters which fertilize four different valleys."

Such are the essential facts of electrolytic dissociation.

Among the numerous developments of the theory announced by Arrhenius in 1887 none is of more general practical importance than the resolution of "acidity" into two components—the concentration of the hydrogen ions, and the quantity of acid capable of furnishing this ionized hydrogen. For two reasons the hydrogen ion occupies a unique place in the estimation of students of ionization. First, it is a dissociation product of the great majority of compounds of biochemical importance. Second, it is the ion for which methods of determination have been best developed. Its importance and its mensurability have thus conspired to make it a center of interest. The consequent grouping of phenomena about the activity of the hydrogen ion is unfortunate when it confers undue weight upon a subordinate aspect of a problem or when it tends to obscure possibilities of broader generalization. Nevertheless, such grouping is often convenient, often of immediate value and frequently illuminating. Especially in the field of biochemistry it has coördinated a vast amount of material. It has placed us at a point of vantage from which we must look with admiration upon the intuition of men like Pasteur, who, without the aid of the precise conceptions which guide us, handled "acidity" with so few mistakes.

In the charming descriptions of his experimental work Pasteur has given us glimpses of his discernment of some of the effects of "acidity" in biochemical processes. In the opening chapter of *Studies on Fermentation* he noted that the relatively high acidity of must favors a natural alcoholic fermentation in wine, while the low acidity of wort induces difficulties in the brewing of beer. He recognized the importance of acidity for the cultivation of the bacteria which he discovered and was quick to see the lack of

such an appreciation in his opponents. In describing that process which has come to bear his name Pasteur remarks, "It is easy to show that these differences in temperature which are required to secure organic liquids from ultimate change depend exclusively upon the state of the liquids, their nature and above all upon the conditions which affect their *neutrality whether towards acids or bases.*" The italics, which are ours, emphasize language which indicates that Pasteur was aware of difficulties which were not removed till recently. Had Pasteur, and doubtless others of like discernment, relied exclusively upon volumetric determination of acidity they would certainly have fallen into the pitfalls which at a later date injured the faith of the bacteriologist in the methods of the chemist. Was it reliance upon litmus which aided him? Perhaps the time factor involved in the use of litmus *paper*, which is now held as a grave objection, enabled Pasteur to judge between extremes of reaction which the range of litmus as an indicator in equilibrium does not cover. At all events he recognized distinctions which we now attribute to hydrogen ion concentrations. Over half a century later we find some of Pasteur's suggestions correlated with a marvelous development in biochemistry. The strongest stimulus to this development can doubtless be traced to the work of Sørensen at the Carlsberg Laboratory in Copenhagen and not so much to his admirable exposition of the effect of the hydrogen ion upon the activity of enzymes as to his development of methods. At about the same time Henderson of Harvard, by setting forth clearly the equilibria among the acids and bases of the blood, indicated what could be done in the realm of physiology and stimulated those researches which have become one of the most beautiful chapters in this science.

Today we find new indicators or improved hydrogen electrode methods in the physiological laboratory, in the media room of the bacteriologist, serving the analyst in niceties of separation and the manufacturer in the control of processes. The material which was admirably summarized by Michaelis in 1914, and to which Michaelis himself had contributed very extensively, presents a picture whose significance he who runs may read. There is a vast field of usefulness for methods of determining the hydrogen ion. There is real significance in the fruits so far won.

There remain many territories to explore and to cultivate. We are only at the frontier.

In the meantime it will not be forgotten that our knowledge of the hydrogen ion is an integral part of a conception which has been under academic study for many years and that the time has come when the limitations as well as certain defects are plainly apparent. While there is now no tendency nor any good ground to discredit the theory of electrolytic dissociation in its essential aspects, there is dissatisfaction over some of the quantitative relationships and a demand for broader conceptions. It requires no divination to perceive that while we remain without a clear conception of why an electrolyte should in the first instance dissociate, we have not reached a generalization which can cover all the points now in doubt. Perhaps the new developments in physics will furnish the key. When and how the door will open cannot be foreseen but it is well to be aware of the imminence of new developments that we may keep our data as pure as is convenient and emphasize the experimental material of permanent value. We may look forward to continued accumulation of important data under the guidance of present conceptions, to distinguished services which these conceptions can render to various sciences and to the critical examination of the material gathered under the present régime for the elements of permanent value. These elements will be found in the data of direct experimentation, in those incontrovertible measurements which, though they be but approximations, have immediate pragmatic value and promise to furnish the bone and sinew of future theory. In the gathering of such data guiding hypotheses and coördinating theories are necessary but experimental methods are vital.

The time seems to have come when little of importance is to be accomplished by assembling under one title the details of the manifold applications of hydrogen electrode and indicator methods. It would be pleasing to have in English a work comparable in scope with Michaelis' *Die Wasserstoffionenkonzentration*; but even in the short years since the publication of this monograph the developments in special subjects have reached such detail that they must be redispersed among the several sciences, and made an integral part of these rather than an uncoordinated treatise by themselves. There remains the need for a

detailed exposition, under one cover, of the two *methods* which are in use daily by workers in several distinct branches of biological science. It is not because the author feels especially qualified to make such an exposition that this book is written, but rather because, after waiting in vain for such a book to appear, he has responded sympathetically to appeals, knowing full well from his own experience how widely scattered is the information under daily requisition by scores of fellow workers.

For the benefit of those to whom the subject may be new there is given in the last chapter a running summary of some of the principal applications of the methods. This is written in the form of an index to the bibliography, a bibliography which is admittedly incomplete for several topics and unbalanced in others, but which, it is believed, contains numerous nuclei for the assembling of literature on various topics.

The author welcomes this opportunity to express his appreciation of the broad policy of research established in the Dairy Division Laboratories of the Department of Agriculture under the immediate administration of Mr. Rawl and Mr. Rogers. Their kindness and encouragement have made possible studies which extend beyond the range of the specialized problems to which research might have been confined and it is hoped that the bread upon the waters may return. To Dr. H. A. Lubs is due the credit for studies on the synthesis of sulfonphthalein indicators which made possible their immediate application in bacteriological researches which have emanated from this laboratory. Acknowledgment is hereby made of the free use of quotations taken from the paper *The Colorimetric Determination of Hydrogen Ion Concentration and Its Applications in Bacteriology* published in the *Journal of Bacteriology* under the joint authorship of Clark and Lubs.

The author thanks his wife, his mother, Dr. H. W. Fowle and Dr. H. Connet for aid in the correction of manuscript and proof, and Dr. Paul Klopsteg for valuable suggestions.

It is a pleasure to know that the publication of the photograph of Professor S. P. L. Sørensen of the Carlsberg Laboratory in Copenhagen will be welcomed by American biochemists all of whom admire his work.

Chevy Chase, Maryland
March 17, 1920.

CHAPTER I

INTRODUCTION—SOME GENERAL RELATIONS AMONG ACIDS AND BASES

In a country rich in gold observant wayfarers may find nuggets on their path, but only systematic mining can provide the currency of nations.—F. GOWLAND HOPKINS.

Why certain solvents such as water should cause or permit the splitting of a compound into electrically charged bodies, called ions, has not yet been very clearly explained. That they do has been demonstrated with reasonable certainty. The evidences are described in texts of physical chemistry and will not be reviewed here, except so far as the verification of certain of the laws of electrolytic equilibria furnish evidence. Granting that ionization takes place, we have to formulate certain relations which are of special significance for the subject at hand.

What takes place on electrolytic dissociation may be conveniently pictured as follows. Modern physics has indicated that an element is made up of an aggregate of unit electrical charges called electrons grouped at relatively enormous distances about a central and electrically neutralizing charge of positive electricity. The number of the electrons and their configuration are supposed to distinguish the several elements. Certain of the electrons are less firmly incorporated and constitute the virtual if not the actual elements of primary valency. It is presumably these valence electrons which manifest themselves in electrolytic dissociation, for when a compound such as HCl dissociates, the hydrogen loses a negative charge while the chlorine has gained a negative charge. Apparently an electron has been gained by the chlorine at the expense of the hydrogen. In the case of complex compounds, such as acetic acid, a similar exchange occurs, the group CH_3COO acting as a unit. As a result of losing an electron the hydrogen becomes, relative to the chlorine or acetate group, charged positively and is then a hydrogen ion.

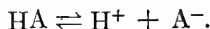
There is reason to believe that the hydrogen ion is unique in that it consists of the unit positive charge alone.

The term ion is generic. It will be recalled that positively charged ions are called cations and negatively charged ions are called anions.

It is the electrical charge or charges which give to ions many of their peculiar properties and which permit the application

of electrical methods to their study. But the electrical charge does not so seriously affect them as to prevent the application of the laws of equilibrium first discovered in the study of other substances.

If, for instance, we have an acid of the type HA and its dissociation into H^+ and A^- is reversible, we may express the reversible reaction as follows:



Then applying the mass law and representing concentration by brackets we have:

$$\frac{[H^+] \times [A^-]}{[HA]} = K_a \quad (1)$$

K_a is the so called ionization or dissociation constant.

Equation (1) is of fundamental importance to the following treatment. Expressed in words it says that for any given acid the product of the concentrations of anion and hydrogen ion (cation) divided by the concentration of that portion of the acid which remains undissociated (dissociation residue) is a constant. Since ionization involves the mutual relationship of solvent and solute, the dissociation constant of an acid may vary considerably with the solvent; but since we shall confine our attention to water as a solvent we may consider its influence constant at least for dilute solutions, and, with due regard for the influence of temperature, we may take the dissociation constant of a particular acid as a characteristic number.

From equation (1) it will be seen that if K_a is large there must be a relatively large proportion of the products of dissociation and *vice versa* when K_a is small. If the extent of the dissociation of an acid when left to itself in solution be taken as a measure of the "strength" of the acid then K_a is a measure of such "strength."

In a similar way the dissociation of a base, for instance one of the type BOH dissociating as $BOH \rightleftharpoons B^+ + OH^-$, may be represented in equilibrium by

$$\frac{[B^+] \times [OH^-]}{[BOH]} = K_b \quad (2)$$

Just as K_a is characteristic of an acid so is K_b characteristic of a base.

A very important relationship between acids and bases in aqueous solution is brought about by the conduct of water. It dissociates into the hydrogen ion (H^+) characteristic of acids and an ion characteristic of bases which is generally considered to be OH^- , called the hydroxyl ion. The equilibrium of the reversible reaction $HOH \rightleftharpoons H^+ + OH^-$ is represented by

$$\frac{[H^+] \times [OH^-]}{[HOH]} = k$$

Because the concentration of the undissociated water is so large in relation to the dissociation products, k may be replaced by another constant and the above equation written:

$$[H^+] \times [OH^-] = K_w. \quad (3)$$

It follows from the above equation that, no matter how concentrated the hydroxyl ions may be, there must remain sufficient hydrogen ions to satisfy the above relation.¹ This permits us to speak of the hydrogen ion concentration of alkaline solutions and to construct a scale of acidity-alkalinity in which we do not discriminate between hydrogen and hydroxyl ions.

Starting from equations (1), (2) and (3), applying certain approximations and then using graphic methods of presentation we can present a generalized picture of the conduct of acids and bases similar to that first used by Henderson (1908). The final simplicity of the picture warrants what may at first appear to be a complicated reconstruction of the above equations.

In order to emphasize the hydrogen ion concentration as the quantity in equation (1) with which the other species keep in adjustment, let us rewrite equation (1) as follows:

$$\frac{1}{[H^+]} = \frac{[A^-]}{K_a[HA]}$$

¹ $K_w = 10^{-14}$. If in an alkaline solution the concentration of hydroxyl ions is 0.01 normal (10^{-2}), $[H^+] = \frac{K_w}{[OH^-]} = \frac{10^{-14}}{10^{-2}} = 10^{-12}$ N.

We choose the form which will give the reciprocal of $[H^+]$ because we shall have to make use of the logarithm of this value under the symbol pH for reasons which will appear later. For the present let it be granted that it will be found convenient to use $\log \frac{1}{[H^+]}$ rather than $[H^+]$. Taking the logarithm of each side of the above equation we have

$$\log \frac{1}{[H^+]} = \log \frac{1}{K_a} + \log \frac{[A^-]}{[HA]} \quad (4)$$

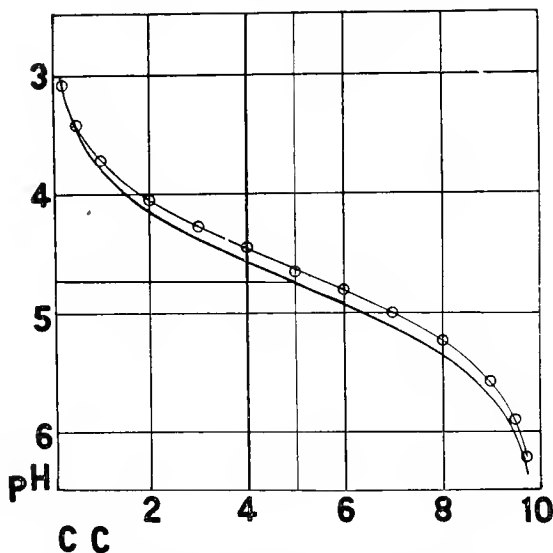


FIG. 1. COMPARISON OF EXPERIMENTAL TITRATION CURVE OF ACETIC ACID WITH THEORETICAL APPROXIMATION

With the use of this equation we can chart some important relationships. Let it first be applied to what may be called "titration curves."

Suppose we titrate 10 cc. of 0.2N acetic acid with 0.2N sodium hydroxid. Ordinarily no attention would be given to the state of the solution until the so called "end point" of the titration were reached. In the present instance we shall follow the course of the titration from the beginning by determining after each addition of alkali the hydrogen ion concentration.

The experimental curve is plotted in figure 1. Let us compare it with the values obtained by the use of equation (4).

In the first place acetic acid is classed among the moderately weak acids. Its dissociation constant as given in Landolt-Börnstein is 1.82×10^{-5} at 18°C . Hence $\log \frac{1}{K_a} = 4.74$. Because of the small dissociation of acetic acid (less than 2 per cent in 0.2N solution even with no acetate present) the concentration of the undissociated residue [HAc] is *approximately* equal to the concentration of the total acetic acid. It is characteristic of the alkali salts of acids that they are very highly dissociated. Therefore, when sodium hydroxid is added to the acetic acid solution, the resulting sodium acetate furnishes the greater amount of the total acetate (Ac^-) ions. As an approximation therefore we may substitute for the ratio $\frac{[\text{A}^-]}{[\text{HA}]}$ in equation (4) the ratio $\frac{[\text{salt}]}{[\text{acid}]}$.

TABLE 1

Comparison of $\log 1/[\text{H}^+]$ for acetic acid-sodium acetate calculated by means of the approximation formulated in equation (5) and determined experimentally by Walpole

N/5 NaOH	RATIO [SALT] [ACID]	LOG RATIO	LOG $1/K_a$	LOG $1/[\text{H}^+]$ CALCULATED	LOG $1/[\text{H}^+]$ WALPOLE
cc.					
0.20	0.020	-1.69	4.74	3.05	3.08
0.25	0.026	-1.59	4.74	3.15	3.15
0.30	0.031	-1.51	4.74	3.23	3.20
0.40	0.042	-1.38	4.74	3.36	3.32
0.50	0.053	-1.28	4.74	3.46	3.42
0.75	0.081	-1.09	4.74	3.65	3.59
1.0	0.111	-0.95	4.74	3.79	3.72
2.0	0.250	-0.60	4.74	4.14	4.05
3.0	0.429	-0.37	4.74	4.37	4.27
4.0	0.667	-0.18	4.74	4.56	4.45
5.0	1.000	0.00	4.74	4.74	4.63
6.0	1.500	+0.18	4.74	4.92	4.80
7.0	2.33	+0.37	4.74	5.11	4.99
7.5	3.00	+0.48	4.74	5.22	5.09
8.0	4.00	+0.60	4.74	5.34	5.23
8.5	5.67	+0.75	4.74	5.49	5.37
9.0	9.00	+0.95	4.74	5.69	5.57
9.5	19.00	+1.28	4.74	6.02	5.89
9.625	25.67	+1.41	4.74	6.15	6.02
9.75	39.00	+1.59	4.74	6.33	6.21
9.875	79.00	+1.90	4.74	6.64	6.52

Equation (4) then becomes:

$$\log \frac{1}{[\text{H}^+]} = \log \frac{1}{K_a} + \log \frac{[\text{salt}]}{[\text{acid}]} \quad (5)$$

In table 1 are given the ratios $\frac{[\text{salt}]}{[\text{acid}]}$ calculated from the number of cubic centimeters of 0.2N alkali added to 10 cc. of 0.2N acetic acid. Then follow the logarithms of these ratios, the value of $\log \frac{1}{K_a}$ for acetic acid, and $\log \frac{1}{[\text{H}^+]}$ calculated from these data by means of equation (5). Finally in the last column are given the values of $\log \frac{1}{[\text{H}^+]}$ calculated by Walpole (1914) from his hydrogen electrode measurements. The experimental values $\text{pH} = \log \frac{1}{[\text{H}^+]}$ are plotted in figure 1 as circles while the values calculated by means of the approximation equation (5) are on the unmarked line. There is evidently a substantial agreement with a more or less regular discrepancy which remains to be explained. The discrepancy is due in large measure to the assumption that the salt is wholly dissociated and that it is entirely responsible for the anions of equation (4). If there be applied a correction for the partial dissociation of the acetate, there is obtained a much closer agreement.

But even this correction does not take into consideration certain minor points, and it leaves untouched the accuracy with which K has been determined and the comparability of data obtained by widely different methods which are often applied in making such calculations as those indicated above.

We shall proceed with the approximate treatment to bring out certain more general relations, and shall leave to Chapter XIX their further application to ordinary titrations.

Inspection of the titration curve of figure 1 will show that along what we may call the flat portion of the curve considerable alkali has to be added to produce much change in $\log \frac{1}{[\text{H}^+]}$ or (pH). Conversely the addition of a strong acid would not have anywhere near the effect at this flat portion of the curve that it

would have near either end. It is evident that it is only within a certain zone of $\log \frac{1}{[H^+]}$ that a mixture of an acid with its salt produces a stabilized hydrogen ion concentration or pH. Mixtures lying within such a zone are called regulator mixtures and the generic technical term covering all such cases where the hydrogen ion concentration is stabilized against added acid or alkali is "buffer action."

In equation (5) when the ratio

$$\frac{[\text{salt}]}{[\text{acid}]} = 1, \log \frac{1}{[H^+]} = \log \frac{1}{K_a} \text{ or } [H^+] = K_a.$$

In other words the middle portion of the titration curve of a particular acid lies at ("near" if we are to be strict) a point where the hydrogen ion concentration is numerically equal to the dissociation constant.²

Thus if one wishes a solution of $[H^+] = 1 \times 10^{-5}$, an acid with dissociation constant close to this value is selected and mixed with the proper amount of its alkali salt.

Or to look at the matter from another point of view, if we determine the half transformation point in the titration of a weak acid, we know approximately the dissociation constant of the acid.

A similar set of relationships can be constructed for bases.

Instead of putting the fundamental equation (1) into the form which we have utilized in following titration curves it is sometimes advantageous to use the following development.

Transforming (1) we have:

$$\frac{[A^-]}{[HA]} = \frac{K_a}{[H^+]}$$

Now let us represent the acid in whatever form it occurs, whether free or in the form of a salt, by S. Then the undissociated portion $[HA]$ will be $S - [A^-]$. Hence,

² There is implied in this the maintenance of the customary units of concentration. Cf. page 25.

$$\frac{[A^-]}{S - [A^-]} = \frac{K_a}{[H^+]}$$

or

$$\frac{[A^-]}{S} = \frac{K_a}{K_a + [H^+]}$$

The ratio $\frac{[A^-]}{S}$ is the ratio of the dissociated acid to the total acid present in the solution in whatever form. This ratio may be represented by α . Hence,

$$\alpha = \frac{K_a}{K_a + [H^+]} \quad (6)$$

Since we are interested in $\log \frac{1}{[H^+]}$ or pH rather than $[H^+]$, because of the resultant simplification of charge representations and because of other reasons which will appear later, we may recast equation (6) and taking the logarithm of each side we have:

$$\log \frac{1}{[H^+]} = \log \frac{1}{K_a} + \log \frac{\alpha}{(1 - \alpha)} \quad (7)$$

Plotting $\log \frac{1}{[H^+]}$, which is pH, against α as percentage dissociation rather than as a ratio, we obtain the curves A and B of figure 2. These curves are identical in form, the form being determined by the ratio $\frac{\alpha}{(1 - \alpha)}$.³ Their position on the pH axis is determined by the value of the dissociation constant in the expression $\log \frac{1}{K_a}$.

In a similar way we arrive at the relation for bases:

$$\alpha = \frac{K_b}{K_b + [OH^-]} \quad (8)$$

or

$$\log [OH^-] = \log \frac{K_b (1 - \alpha)}{\alpha}. \quad (9)$$

³ Since (7) is useful in plotting type curves a table of values for $\log \frac{\alpha}{1 - \alpha}$ is given in the appendix, p. 306.

But since we wish to deal uniformly with $\log \frac{1}{[H^+]}$, which is pH, rather than with the hydroxyl ion concentration or any direct function thereof, we shall introduce the water equilibrium, equation (3). Then (9) becomes

$$\log \frac{K_w}{[H^+]} = \log \frac{K_b (1 - \alpha)}{\alpha}$$

or

$$\text{pH} = \log \frac{1}{[H^+]} = \log \frac{K_b}{K_w} + \log \frac{(1 - \alpha)}{\alpha} \quad (10)$$

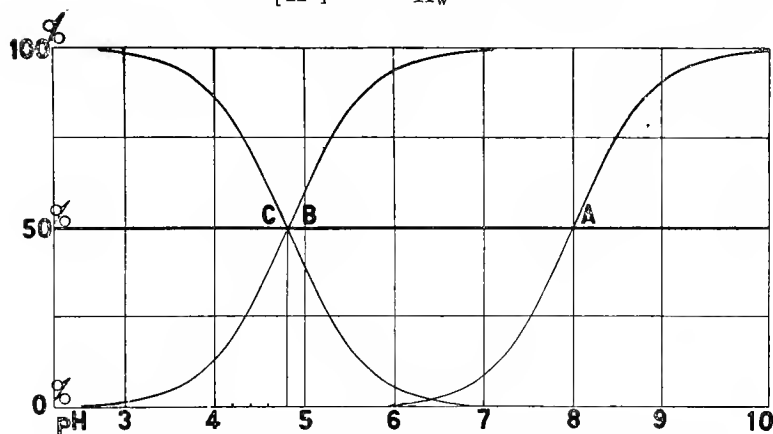


FIG. 2. DISSOCIATION CURVES AND DISSOCIATION RESIDUE CURVES

A. Dissociation curve of acid, $\log \frac{1}{K_a} = 8.0$.

B. Dissociation curve of acid, $\log \frac{1}{K_a} = 4.8$.

C. Dissociation residue curve of acid, $\log \frac{1}{K_a} = 4.8$, or dissociation curve

of a base $\log \frac{1}{K_b} = \log \frac{1}{K_w} - 4.8$.

With the introduction of K_w , the dissociation constant of water, into our equations it becomes advisable to consider its numerical value. K_w has been determined in a variety of ways of which the following are examples. Kohlrausch and Heydweiller (1894) determined the electrical conductivity of extremely pure water.

Assuming that the conductance is due to the mobility of the hydrogen and the hydroxyl ions, and that these are present in equal concentration, their product is found to be 1.1×10^{-14} . The hydrolysis of methyl acetate having been found to be proportional to the concentration of hydroxyl ions, Wijs (1893) determined the hydrolysis by water and found $K_w = 1.44 \times 10^{-14}$.

By determining the hydrogen ion concentration with the hydrogen electrode in solutions of known hydroxyl ion concentration (as determined by conductance measurements), K_w is obtained from the product of the concentrations of the two ions. With this method Lewis, Brighton and Sebastian (1917) found the value 1.012×10^{-14} at 25°C .

Since in pure water $[\text{H}^+] = [\text{OH}^-]$, $[\text{H}^+]$ or $[\text{OH}^-] = \sqrt{K_w}$. Hence from the datum of Lewis, Brighton and Sebastian the normality of H^+ or OH^- in pure water at 25°C . is $\sqrt{K_w} = 1.006 \times 10^{-7}$ (practically $\text{pH} = 7.0$).

Introducing the numerical value of K_w into equation (10) we have the convenient form:

$$\text{pH} = 14 - \log \frac{1}{K_b} + \log \frac{(1 - \alpha)}{\alpha} \quad (11)$$

In figure 2 we have plotted α as percentage dissociation. It is obvious that the percentage dissociation residue will give the complement of the type curve and will cross any particular one of these at the fifty per cent dissociation point. See, for example, the curve C of figure 2.

Now by comparing equation (7) with equation (11) it is found that the curve for the dissociation residue of an acid is identical with the curve for the dissociation of a base when K_a of the acid is related to K_b of the base as $\log \frac{1}{K_a} = 14 - \log \frac{1}{K_b}$. In other words curve C (fig. 2) is either the dissociation residue curve of an acid for which $\log \frac{1}{K_a} = 4.8$ or the dissociation curve of a base for which $\log \frac{1}{K_b} = 9.2$ since $(14 - \log \frac{1}{K_b} = 4.8)$.

The importance of this relation lies in the fact that a determination of the effect of hydrogen ion concentration on some process may not reveal whether the phenomenon has to do with an acid or a base, unless an independent method reveals the nature of the active substance.

The following values of $\log \frac{1}{K_w}$ given by Michaelis (1914) were obtained on a somewhat different basis from that used by Lewis, Brighton and Sebastian (1917).

TEMPERATURE	$\log \frac{1}{K_w}$	pH OF NEUTRAL POINT
16	14.200	7.10
17	14.165	7.08
18	14.130	7.07
19	14.100	7.05
20	14.065	7.03
21	14.030	7.02
22	13.995	7.00
23	13.960	6.98
24	13.925	6.96
25	13.895	6.95
26	13.860	6.93
27	13.825	6.91
28	13.790	6.90
29	13.755	6.88
30	13.725	6.86
31	13.690	6.85
32	13.660	6.83
33	13.630	6.82
34	13.600	6.80
35	13.567	6.78
36	13.535	6.77
37	13.505	6.75
38	13.475	6.74
39	13.445	6.72
40	13.420	6.71

The treatment accorded simple acids and bases may be extended to poly-acidic acids and poly-basic bases as well as to those compounds containing both acidic and basic groups which are called amphoteric electrolytes. It seems to be true very often for such compounds that they dissociate in steps as is illustrated

in the titration curve of the tri-acidic phosphoric acid shown on page 32. In this, as in many other cases, the several dissociation constants are of such widely different magnitudes that, when we plot the dissociation curves as if of separate acids possessing these dissociation constants, the curves do not seriously overlap.

Such acids may therefore be treated as if composed of two independent acids. The effect produced when two dissociation constants lie closer together is illustrated by the titration curve of o-phthalic acid shown on page 191.

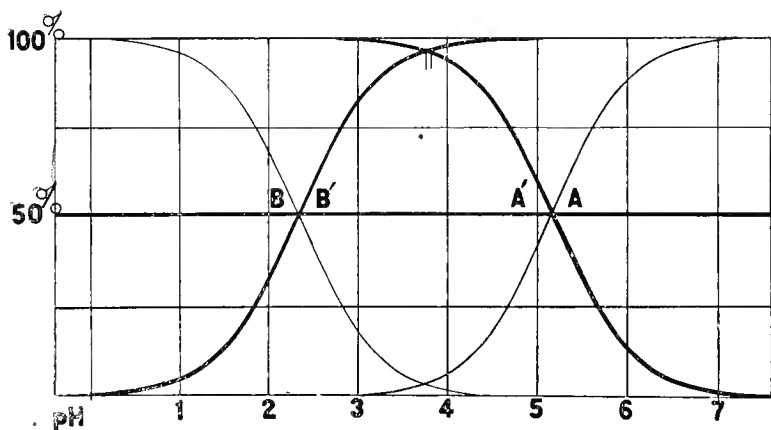


FIG. 3. DISSOCIATION AND DISSOCIATION RESIDUE CURVES OF p-AMINO-BENZOIC ACID

Treated as if the amphoteric electrolyte were composed of an acid of $\log \frac{1}{K_a} = 5.17$ and a base of $\log \frac{1}{K_b} = \log \frac{1}{K_w} - 2.36$.

For amphoteric electrolytes (i.e., electrolytes containing acidic and basic groups) a relation of great importance to protein chemistry may be illustrated by the conduct of a simple ampholyte, p-amino benzoic acid. The acid dissociation constant K_a is 6.3×10^{-6} and the basic dissociation constant K_b is 2.3×10^{-12} (Scudder). Translating these into the corresponding pH values we have 5.17 and 2.36. If we regard the compound as if it were made up of an acid and a base with the above dissociation constants (in terms of pH) and each independent of the other, we can plot the dissociation curves of each with the aid of equations

(7 and 11). In each case the dissociation residue curves are the complements. These are plotted in figure 3 with heavy lines. It is seen that they cross at $\text{pH} = 3.78$. This means that at $\text{pH} = 3.78$ there is a maximum of undissociated residue. Now if the salts are more soluble than the free compound itself there should be a minimum solubility at $\text{pH} 3.78$. Michaelis and Davidsohn (1910) found a minimum solubility at $\text{pH} 3.80$. As Michaelis and others have shown, such points, called the *isoelectric points*, as they are found for the amphoteric proteins, are points of optimum precipitation, minimum solubility, minimum viscosity, minimum swelling, optimum agglutination, etc.

When the dissociation constants of a simple amphoteric electrolyte are known, the isoelectric point, I , may be calculated from the relation

$$I = \sqrt{\frac{K_a}{K_b} K_w}.$$

Only in case $K_a = K_b$ will the isoelectric point correspond with "neutrality" as was once supposed.

THE pH SCALE

When "acidity" was resolved into its two components the normality unit was retained for each. As a normal solution of an acid had been defined as one containing in 1 litre of solution the equivalent of 1 gram atom of acidic hydrogen, so the normal solution of the hydrogen ion was defined to be one containing in 1 litre of solution 1 gram atom of hydrogen ions.⁴

To distinguish between these two components with their common unit it has been suggested that we call "normality" in its older sense the *quantity* factor of "acidity" and the hydrogen ion concentration the *intensity* factor. This may serve to emphasize a distinction, but the suggested analogy with the quantity and intensity factors of energy is confusing when we retain for each a unit of the same category. Nevertheless the two components remain in a restricted sense the quantity and intensity factors of "acidity." The one is the total quantity of available acid. The

⁴ It makes little difference whether the atomic weight of hydrogen be taken as 1.008 or as 1.0 in calculating $[\text{H}^+]$.

second, the concentration of the hydrogen ions, represents the real intensity of "acidity" whenever it is the hydrogen ion which is the more directly active participant in a reaction. This is admirably expressed when we use for hydrogen ion concentrations a mode of expression which links it with the *potential* of a hydrogen electrode. It so happens that in determining the hydrogen ion concentration with the hydrogen electrode the potentials of this electrode are put into an equation which reduces to the form:

$$\frac{\text{Potential}}{\text{Numerical factor}} = \log \frac{1}{[\text{H}^+]}$$

Thus $\log \frac{1}{[\text{H}^+]}$ is at once obtained by the most simple of calculations. Sørensen (1909) saw that this value serves to define a hydrogen ion concentration quite as well as $[\text{H}^+]$ itself and in his *Enzyme Studies II*, he used this mode of expression and gave to $\log \frac{1}{[\text{H}^+]}$ the symbol P_{H^+} .

As a matter of typographical convenience⁵ we shall adopt pH in place of P_{H^+} . Since this is coming into wide usage its uniform adoption is recommended in place of the bothersome variations which have made their way into the literature.

The convenience of pH over $[\text{H}^+]$ is manifest when we consider the values of $[\text{H}^+]$ encountered in physiological solutions. The hydrogen ion concentration, $[\text{H}^+]$, of blood is about 0.000,000,04N. The limit for many bacteria is near 0.000,01N. Though convenient abbreviations of these unwieldy figures are 4×10^{-8} and 1×10^{-5} , there remains the difficulty of plotting such values on ordinary cross-section paper. To show a difference between 1×10^{-8} and 3×10^{-8} the chart would have to be extended out of bounds to include 1×10^{-4} . As was shown in previous pages it is the logarithmic charting of $[\text{H}^+]$ which brings out the fact that the difference between 1×10^{-8} and 3×10^{-8} may be of as great an importance for one set of equilibria as the enormously greater numerical difference between 1×10^{-4} and 3×10^{-4} is for another set of equilibria.

⁵ As is the custom of the *Journal of Biological Chemistry*.

In Chapter XVII another good reason will be given for adhering to the use of pH; but at this point it may be well to mention that not only are the errors of the two chief methods of determination proportional to pH but also that pH, being a linear function of hydrogen electrode potentials, is representative of several important relations which cannot be foreseen by those who regard it *merely* as $\log \frac{1}{[H^+]}$.

For those who prefer to use $[H^+]$, the relation of $[H^+]$ to pH may be illustrated as follows.

$$\text{If } [H^+] = 2 \times 10^{-4}N, \log \frac{1}{[H^+]} = \log \frac{1}{2 \times 10^{-4}} = \log 5,000 = 3.699$$

From pH or $[H^+]$ the corresponding $[OH^-]$ may be found from the relation $[OH^-] = \frac{K_w}{[H^+]}$. K_w varies with the temperature. If the rounded value 10^{-14} be used $\frac{1}{[OH^-]} = \text{antilog } (14 - \text{pH})$.

In the appendix will be found a section of a table showing the relation of pH to $[H^+]$ and constructed so as to be of general use for all ranges of either quantity.

A more elaborate table in which are included hydrogen electrode potentials has been published by Schmidt and Hoagland (1919)⁶ and Matula (1916).

It is unfortunate that a mode of expression so well adapted to the treatment of various relations should conflict with a mental habit. $[H^+]$ represents the hydrogen ion concentration, the quantity usually thought of in conversation when we speak of increases or decreases in acidity. pH varies inversely as $[H^+]$. This is confusing.

The normality mode of expression has historical priority and consequently conventional force. Since there is a hydrogen ion concentration for each hydroxyl ion concentration it became the custom, following Friedenthal (1904), to express both acidities and alkalinities in terms of $[H^+]$. This gave a scale of one denomination and the meaning of "higher" and of "lower" became firmly fixed. Now we meet the new scale with its direction reversed. The inconvenience is unquestionable and very largely because of it the pH scale has been seriously criticized. The opposition has

⁶ In using the tables of Schmidt and Hoagland the various values used by these authors should be noted. Cf. discussion in Chapter XVII.

taken its most definite form in a proposal by Wherry (1919). Wherry suggests that instead of the present scale, centered at normal hydrogen ion concentration, there be a specific acidity-specific alkalinity scale with its unit the gram equivalents of H^+ and OH^- in pure water. Using a logarithmic function similar to pH which he calls X_H , Wherry obtains a scale in which X_H 0, 1, 2, -1, -2, etc., correspond respectively to pH 7, 6, 5, 8, 9, etc. provided pH 7 is considered the true neutral point at all temperatures.

Wherry's purpose is very frankly stated to be the convenience of "workers in non-mathematical sciences." If the actual physical and mathematical derivation of X_H is to be neglected, Wherry's scale offers some convenience to "the worker in the non-mathematical sciences." If, however, it is to be taken seriously by those who are interested in keeping even the cruder data as close as is convenient to actual experimental measurements and in keeping uniformity in the mode of expressing such data, then the use of X_H is open to serious objections. The present pH scale is to some extent arbitrary as is shown in Chapter XVII. It does however express with some directness the relation of the scale to the conduct of the hydrogen electrode, upon which the system is calibrated. It does not involve any assumption regarding the nature of that pure water which is never used, which seldom is considered in calculations, whose importance has been overemphasized and which is yet the standard of Wherry's scale. As directly derived from the potential of the normal hydrogen electrode, which is generally taken as the standard for both hydrogen electrode and all electrode potentials, pH values are useful where X_H values are not. Since pH is thus derived it contains implicitly a direct function of the dissociation constants of acids and bases, whose values as now known involve the use of normality concentrations. The advantage of this was indicated in a previous section of this chapter. In addition there remains the fact that factory workmen, who have never been taught to reckon every relation between acids and bases from the theoretical neutral point, gain a correct picture of the relation of the pH scale to what happens at the significant pH for the process under control, and are not bothered by the direction of the scale. These arguments when considered beside the fact that quantities of important data are already recorded in terms of pH make the admitted convenience of X_H dubious. To have two scales with numerical values so similar might produce a confusion such as was threatened when Friedenthal (1910) expressed hydrogen ion concentrations in terms of grams per cubic centimeter. Cases of the resulting confusion are to be found.

There remains, of course, the argument that for the sake of a restricted convenience an arbitrary scale may be set up and that this scale can be subjected to as refined a treatment as the present scale if readjustment be made all along the line. Is it worth while?

THE EFFECT OF DILUTION

A litre of normal acid becomes a fifth normal solution if diluted to 5 litres; the hydrogen ion concentration may in many instances be affected too little for the change to be detected by any but refined methods. This apparent anomaly is frequently encountered and sometimes advantage of it is taken in the dilution of solutions otherwise too dense optically for the application of the indicator method. The effect of dilution upon the hydrogen ion concentration of a solution may be briefly generalized by some approximations.

Consider an acid of the type HA for the dissociation of which we have the equilibrium equation:

$$\frac{[\text{H}^+] \times [\text{A}^-]}{[\text{HA}]} = K_a$$

If the acid alone is present in the solution we may assume that $[\text{A}^-] = [\text{H}^+]$. Also if $S_a =$ the total acid, $[\text{HA}] = S_a - [\text{H}^+]$.

Substituting these in the above equation and solving for $[\text{H}^+]$ we have:

$$[\text{H}^+] = \sqrt{K_a S_a + \frac{K_a^2}{4}} - \frac{1}{2} K_a$$

When K_a is small in relation to S_a

$$[\text{H}^+] \cong \sqrt{K_a S_a}$$

On these assumptions the hydrogen ion concentration should vary with dilution of the solution (diminution of S_a) only as the square root of $K_a S_a$.

If there is present a salt of the acid we can apply the equation derived on page 18 which shows that the hydrogen ion concentration of a mixture of a weak acid and its highly dissociated salt is determined approximately by the ratio of acid to salt. Since dilution does not change the ratio, such a mixture should not suffer a change of hydrogen ion concentration beyond the narrow limits set by the approximate treatment with which this relation was derived.

Therefore, except for solutions of high hydrogen ion concentration induced by the presence of unneutralized strong acids, the

hydrogen ion concentration should vary with dilution somewhere between the zero change indicated by the last approximation and the square root relation first indicated.

Such a conclusion is limited to those solutions which are buffered in the region of ordinary physiological study and takes no account of changes of equilibrium which sometimes occur in colloidal solutions.

For bases and amphoteric electrolytes similar relations may be deduced.

One or two actual cases may be of interest.

Sørensen has given the following table of the pH values of different dilutions of asparagine and glycocoll.

MOLECULAR CONCENTRATION OF GLYCOCOLL	pH	MOLECULAR CONCENTRATION OF ASPARAGINE	pH
1.0	6.089	1.0	2.954
0.1	6.096	0.1	2.973
0.01	6.155	0.01	3.110
0.001	6.413	0.001	3.521
0.0001	6.782	0.0001	4.166

The dilution here is ten-fold at each step, yet the increase in pH is very small while the solutions are as concentrated as 0.1-0.01 M.

Walpole (1914) besides giving data on the hydrogen electrode potentials of various dilutions of acetic acid and "standard acetate," has determined the effect of a twenty fold dilution of various acetic acid-sodium acetate mixtures. The change of pH on twenty fold dilution of standard acetate is about 0.08 pH and of mixtures of acetic acid and sodium acetate which lie on the flat part of the curve the change of pH with dilution is of the same order of magnitude. When the ratio $\frac{\text{acetic acid}}{\text{sodium acetate}}$ reaches 19/1 the change is about 0.3 pH.

BUFFER ACTION

If we were to add to 1 liter of perfectly pure water of pH = 7.0, 1 cc. of 0.01N HCl, the resulting solution would be about pH =

5.0 and very toxic to many bacteria. If, on the other hand, we were to add this same amount of acid to a liter of a standard beef infusion medium of $\text{pH} = 7.0$, the resulting change in pH would be hardly appreciable. This power of certain solutions to resist change in reaction was commented upon by Fernbach and Hubert (1900) who likened the resistance of phosphate solutions to a "tampon." The word was adopted by Sørensen (1909) and in the German rendition of his paper it became "puffer" and thence the English "buffer." There has been some objection to this word so applied but it has now acquired a clear technical meaning and is so generally used that it should probably be retained. By buffer action then we mean the ability of a solution to resist change in pH through the addition or loss of acid or alkali. This may be illustrated by titration curves such as those shown in figures 4, 5 and 6. The construction of such curves may be illustrated by the following example.

A 1 per cent solution of Witte peptone was found to have a pH value of 6.87. To equal portions of the solution were added successively increasing amounts of 0.1N lactic acid and the resulting pH was measured in each case. There were also added to equal portions of the solution successively increasing amounts of 0.1N NaOH and the resulting pH was measured in each case. The pH values were then plotted on cross section paper as ordinates against the amount of acid or alkali added in each case as abscissas. This gave the curve shown in figure 4. The other curve shown in this figure was constructed with data obtained with a 5 per cent solution of Witte peptone. The curves of figures 5 and 6 were obtained in a similar way.

These curves illustrate the following points. Figure 4 shows that the buffer action of a solution is dependent upon the concentration of the constituents. The 5 per cent solution is much more resistant to change in pH than the 1 per cent solution. It will also be noticed that in either case the buffer action is not the same at all points in the curve. In other words the buffer action can not be expressed by a constant but must be determined for each region of pH . This is illustrated even more clearly by the titration curve for phosphoric acid (fig. 5). At the point where the solution contains only the primary phosphate and again where it contains only the secondary phosphate there is very little buffer

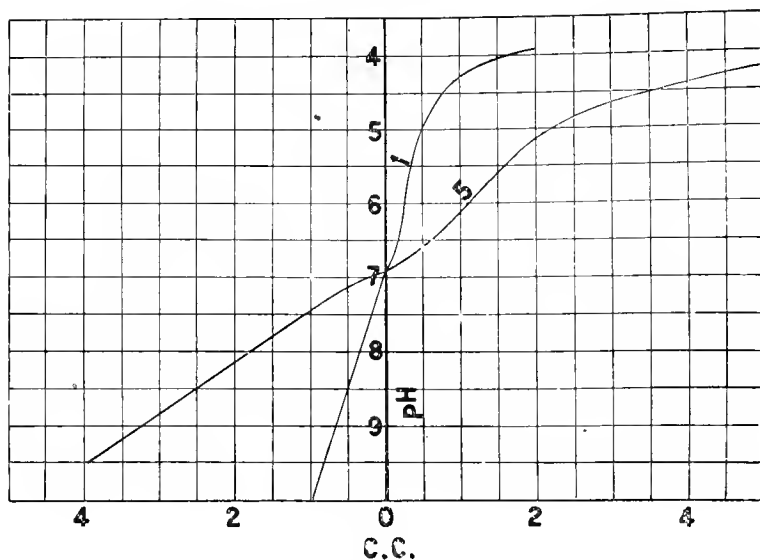


FIG. 4. TITRATION CURVES OF 1 PER CENT AND 5 PER CENT PEPTONE

Ten cubic centimeters of peptone solution titrated with N/10 lactic acid (to right) and with N/10 NaOH (to left).

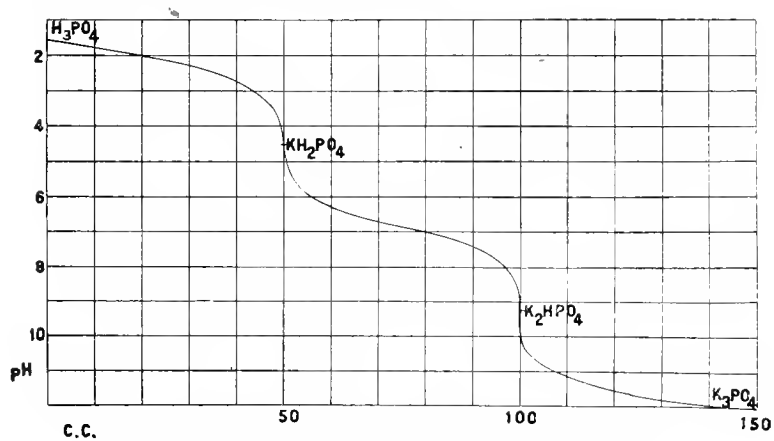


FIG. 5. TITRATION CURVE OF PHOSPHORIC ACID

Fifty cubic centimeters M/10 H_3PO_4 titrated with N/10 KOH.

effect indeed. Furthermore the buffer action of a solution may not be due entirely to the nature of the constituents titrated but also to the nature of the substance with which it is titrated. This point may be illustrated by titrating a beef infusion medium in the one case with hydrochloric acid and in the other case with

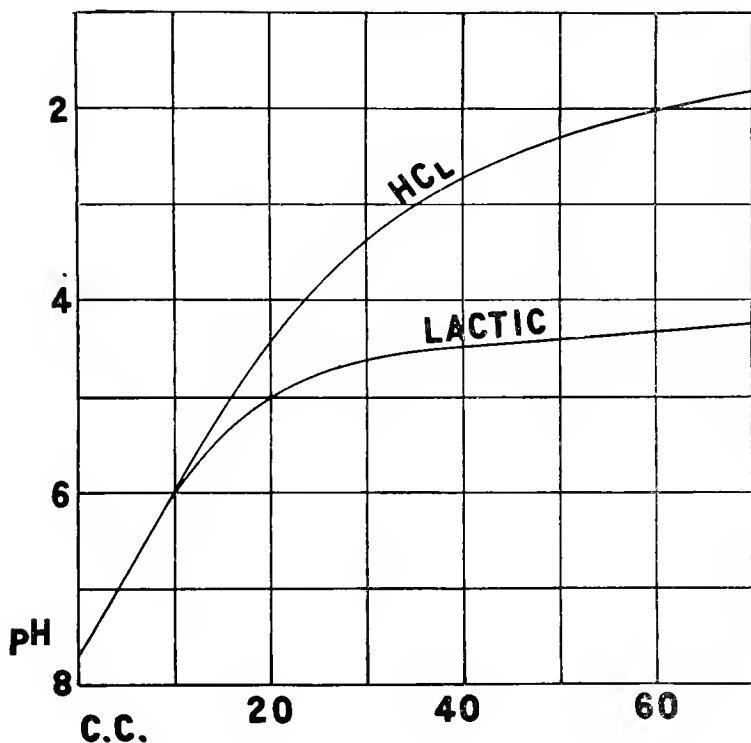


FIG. 6. TITRATION CURVES OF A BEEF INFUSION MEDIUM

One hundred cubic centimeters medium titrated with N/5 HCl and with N/5 lactic acid.

lactic acid, both of the same normality (see fig. 6). It will be seen that at first the two curves are identical. As the region is approached where the dissociation of the "weak" lactic acid is itself suppressed because of the accumulation of lactate ions and the high concentration of the hydrogen ions, further addition of this acid has comparatively little effect. The strongly disso-

ciated hydrochloric acid on the other hand continues to be effective until it, too, at very high hydrogen ion concentrations is suppressed.

These examples will suffice to make it evident that the buffer action of a solution is dependent upon the nature and the concentration of the constituents, upon the pH region where the buffer action is measured and upon the nature of the acid or alkali added. To connect all these variables is a difficult problem. Koppel and Spiro (1914) have attempted to do so but they have necessarily had to leave out of consideration another factor. If there are present any bodies which tend to absorb any of the constituents of a solution which can affect the hydrogen ion concentration of a solution, these bodies will tend to act as buffers or will affect the buffer action of the solution. Henderson (1909) has called attention to this and Bovie (1915) has shown in a very interesting way the buffer action of charcoal. Since some culture media or cultures and many of the solutions whose buffer action must be studied for physiological purposes, contain undissolved or colloidal material which may act in this way, it seems best to consider buffer action in its broadest sense, and to express it by the relative slopes of titration curves determined experimentally. Further illustrations of titration curves of culture media will be found in the papers of Clark (1915) and of Bovie (1915). Titration curves of some inorganic solutions will be found in a paper by Hildebrand (1913). More theoretical treatments of the subject are given in the papers of Henderson (1909), Sørensen (1909), Sørensen (1912), Michaelis (1914) and Koppel and Spiro (1914).

In the regulation of the hydrogen ion concentration of solutions use is frequently made of definite mixtures of acids or bases and their salts. As was shown above this buffer action is strongest near the pH which corresponds to the dissociation constant of the acid or base concerned. This relation permits the selection of acids suitable for a buffer action in any given region by inspection of their dissociation constants.

Unless a solution is buffered to some extent in some way, it is almost impossible to make an accurate electrometric determination of the pH; and because of the influence of traces of carbon dioxid and other acidic or basic contaminations such solutions may be very unsuitable when used for physiological purposes.

Thus the failure to buffer against the effect of so called neutral salts which are not truly neutral may lead to gross error. In like manner the failure to buffer has rendered physiologically unstable certain so called synthetic and supposedly stable culture media.

In the preparation of standard buffer mixtures it is of course, preferable to use a high grade of water if accuracy is required but there is little need of carrying this to an extreme. "Conductivity water" is sometimes specified for the preparation of special standards because the ordinary distilled water of certain regions of the country is such that "distilled water" means nothing. The exercise of judgment is advantageous.

The maintenance of "neutrality" by such solid reagents as calcium carbonate may be considered as a buffer action. It is very important to note however that the use of calcium carbonate may become a grossly inefficient procedure. To show its inefficiency the author has placed at the bottom of a test tube a deep layer of very finely divided, freshly precipitated and well washed calcium carbonate and overlaid this with cultures of bacteria and molds in sugar media. Indicators show that unless the calcium carbonate is frequently and thoroughly shaken with the medium only the solution in direct contact with the calcium carbonate is neutralized. Moulds may develop an acidity as high as pH2 within a few millimeters of the carbonate.

THE CONDUCT OF STRONG ELECTROLYTES

The relations set forth in the preceding pages, even in the approximate form adopted to keep the distinctive lines of the picture clear, afford in their experimental verification the best of evidence that the theory of electrolytic dissociation is essentially correct. That it is incomplete is shown when we turn to the examination of the quantitative data of strong electrolytes—acids such as hydrochloric and nitric and salts such as the simple chlorides. For instance, if the conductance of a solution are ascribed to the concentration and the mobilities of the ions, and if the mobilities be considered constant at all dilutions, the conductance data should satisfy the Ostwald dilution law and furnish a dissociation *constant*. The Ostwald dilution law is $\frac{a^2}{(1-a)v} = k$ where a is the degree of dissociation, v the dilution and k the equilibrium constant which should be independent of the dilution. a should be equal to the ratio of equivalent conductance at dilution v to equivalent conductance calculated for infinite dilution. For potassium chloride, k varies from 0.049 at 1000 dilution to

0.541 at 10 dilution. The discrepancies with hydrochloric acid are comparable.

It is sometimes said that such anomalies disprove the applicability of the mass law. This however is merely a convenient way of saying that certain relations are not sufficiently well known to reveal whether or not the mass law holds.

To give any adequate review of the present status of the problem would require undue space. A most valuable review has recently appeared in the discussions which took place in the Faraday Society and which are published in the December, 1919, number of the *Transactions*. It is there made very evident that the "anomalies" of strong electrolytes have been the bugbear of students of ionization, have stimulated most brilliant researches and promise to be the starting point for new developments which will harmonize the entire body of data.

We are concerned with the conduct of strong electrolytes in this way. Although free acidities of a magnitude that fall within the grosser uncertainties of our knowledge of strong electrolytes are seldom met in physiological solutions, the whole system of pH measurements is scaled from certain assumptions regarding the now uncertain conduct of HCl as will be shown in Chapter XVII. Furthermore we have continually to deal with solutions containing salts whose conduct is so little understood that precise treatment is impossible. This will appear in the so-called salt error of indicators and the strange fact that the *apparent* hydrogen ion concentration as determined with the hydrogen electrode may be raised above the quantity of available acid present by the addition of sufficient salt. To deal with such questions without tracing back through the subtleties of certain tacit assumptions is a most pernicious practice. It seems wiser to admit at once that certain of the more fundamental assumptions are too insecurely based to provide any adequate systematic treatment at the present time, and for this reason such questions as the salt error of indicators will be given in the subsequent chapters what may at first appear to be too brief a treatment. Experimentally the safest procedure to follow whenever the conduct of strong electrolytes enters into the determination of or the use of pH values is standardization of data.

SUPPLEMENTARY REFERENCES

Texts on the principles of electrolytic dissociation: LeBlanc, Jones, Nernst, Ostwald, Stieglitz (1917).

Generalized relations among acids and bases: Henderson (1908), Michaelis (1914), Sørensen (1912).

Symposium on the theory of electrolytic dissociation, especially on the conduct of strong electrolytes. Trans. Faraday Soc., **15**, 1-178, December, 1919.

See also Arrhenius' Faraday Lecture.

CHAPTER II

OUTLINE OF THE COLORIMETRIC METHOD

Acidimetric-alkalimetric indicators are substances whose color in aqueous solution correlates with the hydrogen ion concentration. They may be used in the following manner.

To a series of test tubes are added, seriatim, 10 cc. of each of a series of standard solutions whose pH values are known. Then to each tube are added five drops of indicator solution, the indicator chosen being suitable for the range of pH in use. On mixing indicator and standard there will appear in the tubes a graded series of color.

In ordinary titrations the color of an indicator changes rapidly with the addition of alkali or acid at the "end point" of the titration. In the present instance the standard buffer solutions provide a stabilized pH which holds the color transformation at a particular point. To distinguish this from the changing color observed in titrations we shall adopt Sørensen's term and speak of the *virage* of a particular, stabilized degree of color transformation.

With standards provided, a measurement upon an unknown solution consists simply in adding to 10 cc. of the unknown five drops of the proper indicator and matching the resulting virage with the closest agreeing virage which can be found in the standards. When a color match occurs the standard and the unknown should have the same pH.

The arrangement of standard tubes for comparison purposes is shown in figure 7. This is a simple rack, the test tube holders of which are the clips sold at stationers for holding rubber stamps. A piece of white paper slipped behind the tubes makes a background more satisfactory than the easily stained and irremovable backgrounds sometimes used for such a purpose.

When the virage of an indicator at any pH is well known the standards may be dispensed with for many measurements where precision is unnecessary; but there is no way as satisfactory as the setting up of the standards for the establishment of a correct,

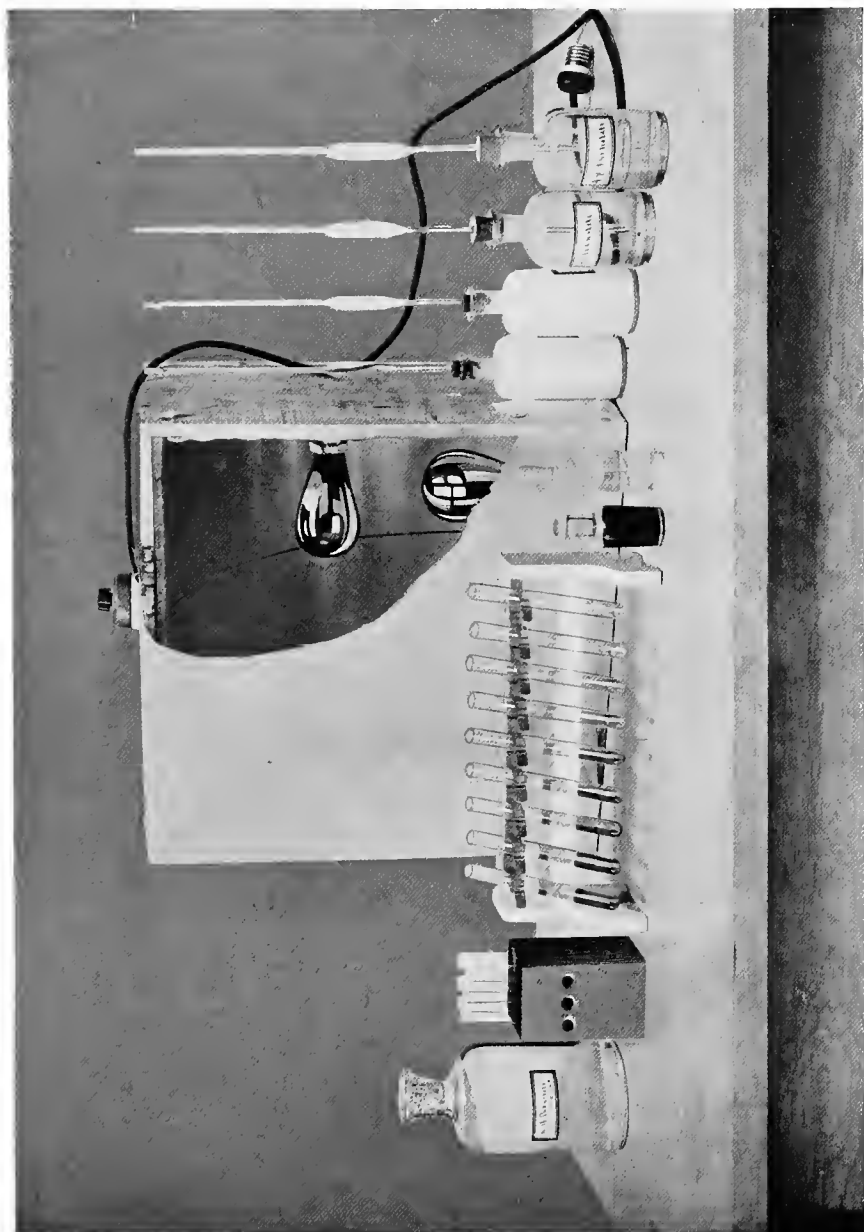


FIG. 7. EQUIPMENT FOR COLORIMETRIC MEASUREMENTS OF pH

vivid and lasting impression of the relations of the various indicators to pH. On the other hand, the author has discovered in his conversations that there are a great many investigators who would like to use indicators for the occasional rough measurement of pH but who are discouraged by a pressure of work which prevents them from taking the time to carefully prepare the standard solutions. To furnish such investigators with a demonstration of the general relations of the various indicators and to furnish *rough* standards the attempt has been made to reproduce the colors in figure 8. The colors of standard buffer solutions containing definite quantities of the several indicators were reproduced very faithfully by Mr. Max Broedel of the Johns Hopkins Medical School. It must be remembered, however, that in undertaking a second reproduction by means of the printer's art the publishers are to be commended for their courage and are not to be held responsible for the inadequacy of the result. Aside from the inherent difficulty in freeing a printed color from the effect of the vehicle, there remains the utter impossibility of reproducing upon paper the exact *virage* observed in a liquid solution. The fundamental phenomena are quantitatively very different in the two cases. Therefore the user of the chart of colors will have to use discretion and some imagination in order to get the real value of the reproductions. If he does not attempt to make them take the place of the standards he should find them useful for class room demonstrations, for refreshing the memory and for rough standards.

In each case the colors were reproduced from tubes 16 mm. internal diameter containing 10 cc. standard buffer solution. The quantities of indicator solution added in each case were as follows: Thymol blue, acid range (T. B. acid range) 1 cc. 0.04 per cent solution. Brom phenol blue (B. P. B.) 0.5 cc. 0.04 per cent solution. Methyl red (M. R.) 0.3 cc. 0.02 per cent solution. Brom cresol purple (B. C. P.) 0.5 cc. 0.04 per cent solution. Brom thymol blue (B. T. B.) 0.5 cc. 0.04 per cent solution. Phenol red (P. R.) 0.5 cc. 0.02 per cent solution. Cresol red (C. R.) 0.5 cc. 0.02 per cent solution. Thymol blue (T. B.) 0.5 cc. 0.04 per cent solution.

The ranges of pH covered by the several indicators in the color chart are:

T. B. (acid range), Thymol blue.....	1.2-2.8
B. P. B., Brom phenol blue.....	3.0-4.6
M. R., Methyl red.....	4.4-6.0
B. C. P., Brom cresol purple.....	5.4-7.0
B. T. B., Brom thymol blue.....	6.0-7.6
P. R., Phenol red.....	6.6-8.2
C. R., Cresol red.....	7.2-8.8
T. B., Thymol blue.....	8.2-9.8

For class room work it is advantageous to show the position of the several indicators on the pH scale by relining each series so that corresponding pH values over-lap.

There are required for the colorimetric method a set of indicators selected for their relative freedom from the so-called protein and salt errors and for their brilliancy. Beside the brilliant and fairly reliable selection of Clark and Lubs there is the carefully studied selection of Sørensen given on page 67 with Sørensen's summary of properties on page 62.

There are also required standard buffer solutions whose pH values are established from hydrogen electrode measurements. It is in the preparation of these standards that the greater part of the labor of the colorimetric method is involved; but, once the stock solutions are carefully made, the preparation of the mixtures is a simple matter. If only the pH range 5.2 to 8.0 is necessary, the Sørensen mixtures of primary and secondary phosphates are the more convenient. If a wider range is desired the system tabulated on pages 75 to 76 is recommended.

For precise measurements there are required constant watchfulness for the several sources of error noted in the following chapter and control by hydrogen electrode measurements. Approximate methods are described in Chapter VII.

In figure 7 are shown several pieces of equipment useful in colorimetric work. Beginning at the left is, first, a sample of a litre bottle used for holding the standard stock solutions, such as M/5 KH Phthalate, which are not seriously affected by exposure to the carbon dioxide of the laboratory air. In Clark and Lubs' series of standards (see page 69) there are required four such bottles. In this same series there is required a container for

standard M/5 NaOH. This should be a paraffined bottle with calibrated burette and soda-lime guard tubes attached.

In figure 7 there is next shown a comparator whose construction is given on page 57. This is used in comparing turbid or colored solutions with the standards. When the turbidity of a tested solution brings into evidence the dichromatism of an indicator as described on page 54, the comparator is used with the light screen shown at the back of figure 7 and described on page 55.

For ordinary colorimetric comparisons the test tube rack shown in the figure and briefly described on page 38 is very useful. Two forms of dropping bottle are next shown and, finally, at the right, two paraffined bottles for alkaline standards and two acid resistant bottles for acid solution. Of such bottles there are required for the series of standards given on pages 75-76 fifty-one bottles and the same number of 10 cc. pipettes. The range of pH thus covered is wider than that called for in special investigations.

The pipettes may have their tips broken to allow quicker delivery of solution without serious violation of volume requirements.

Sørensen's standards, pages 80-82, are designed so that individual 10 cc. samples are made up as required. Larger quantities such as are specified in table 6 provide for the occasional test.

CHAPTER III

THEORY OF INDICATORS

The color change of an indicator cannot fail to excite the wonder of every observer. Even superficial analysis suggests that an explanation requires some knowledge of dye structure, spectroscopy, physiological optics and electrolytic dissociation. Closer study reveals not only the phenomenon of tautomerism but also the necessity for reaching some conception of the manner in which an alteration in the structure of a compound can change electrical relations concerned in the absorption of light. According to the inclination of a reviewer one or another of the manifold phases of indicator theory may be emphasized. We must choose that which is useful to the purpose at hand and include only so much of each phase as will contribute toward avoidance of the more serious mistakes.

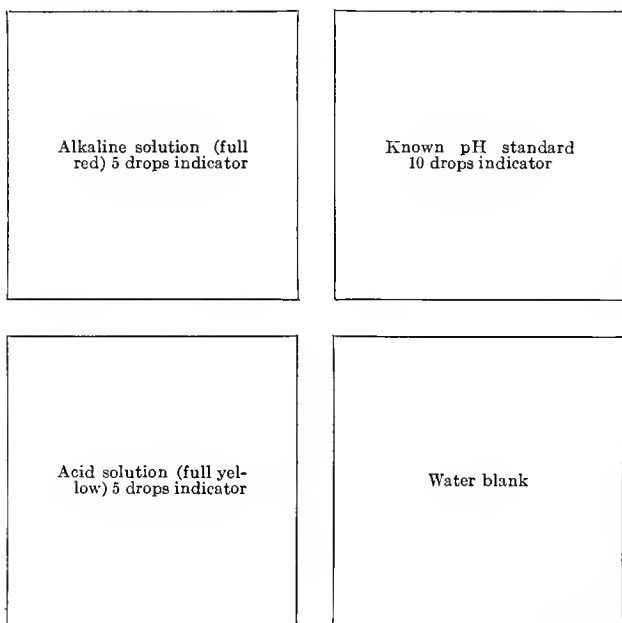
In the first place it should be emphasized that the customary manner of using indicators for the determination of hydrogen ion concentration is basically a comparative method with hydrogen electrode values as "calibration." With standard buffer solutions whose hydrogen ion concentrations have been determined, indicators may be arranged empirically without involving any theory whatsoever. It is well to emphasize this uninspiring, matter-of-fact aspect of the matter because it will remind us that, if so much of practical value has been done without the aid of theory, the application of theory may lead on to greater things.

The first consistent attempt to bring the conduct of indicators into relation with electrolytic dissociation was that of Ostwald (1891). He assumed that indicators are acids or bases whose undissociated molecules have a different color from that of their dissociation products. If this be so, it is evident that the color of an indicator should change with the pH of a solution exactly as the dissociation curves described in Chapter I. If, for instance, the indicator is an acid, colorless in the undissociated form, but colored when dissociated as an anion, then the change of color with the hydrogen ion concentration should conform to the equation:

$$\alpha = \frac{K_a}{K_a + [H^+]}$$

where K_a is the dissociation constant of the acid indicator and α is the degree of dissociation. Assuming then that such a relation does hold, let us determine K_a for a series of indicators in the following way.

From the above equation when $\alpha = \frac{1}{2}$, $K_a = [H^+]$. That is, at a hydrogen ion concentration corresponding numerically to the dissociation constant, the acid is half dissociated. At such a hydrogen ion concentration a colorless-to-red indicator, such as phenolphthalein, should show half the available color; and a yellow-to-red indicator, such as phenol red, should show the half yellow, half red state. We can match this half way state by superimposing two solutions each of a depth equal to the first, if we have in one of the superimposed solutions only the yellow form and in the other only the red form, each concentration equaling half the concentration in the first solution. Such an arrangement is shown diagrammatically in the following figure:



We may not know at the beginning at what pH the half transformation may occur, so we vary the pH of the standard solution until a match with our superimposed solutions does occur. Then we have found, presumably, the hydrogen ion concentration whose numerical value is the dissociation constant of the indicator. Values so obtained by Clark and Lubs (1917) are given in table 2.

TABLE 2
Approximate apparent dissociation constants of indicators

INDICATOR	K	pH
Phenol phthalein.....	2.0×10^{-10}	9.7*
o-Cresol phthalein.....	4.0×10^{-10}	9.4
Carvacrol sulfon phthalein.....	1.0×10^{-9}	9.0
Thymol sulfon phthalein.....	1.2×10^{-9}	8.9
α -naphthol phthalein.....	4.0×10^{-9}	8.4
o-Cresol sulfon phthalein.....	5.0×10^{-9}	8.3
α -naphthol sulfon phthalein.....	5.3×10^{-9}	8.2
Phenol sulfon phthalein.....	1.2×10^{-8}	7.9
Di bromo thymol sulfon phthalein.....	1.0×10^{-7}	7.0
Di bromo o-cresol sulfon phthalein.....	5.0×10^{-7}	6.3
Di propyl red.....	4.0×10^{-6}	5.4
Di methyl red.....	7.9×10^{-6}	5.1†
Tetra bromo phenol sulfon phthalein.....	7.9×10^{-6}	4.1
Thymol sulfon phthalein (acid change).....	2.0×10^{-2}	1.7

* This value is identical with Rosenstein's (1912).

† In the table published in the Journal of the Washington Academy, vol. vi, p. 485, these values for methyl red and propyl red were erroneously interchanged.

Tizard (1910) gives $K_a = 1.05 \times 10^{-5}$ or pH = 4.98 for methyl red considered as an acid.

Gillespie (1920) gives somewhat different values but, since the method used in each case was approximate, the table given above, as it is found in the paper by Clark and Lubs (1917) will do for purposes of illustration. With the aid of the approximately determined apparent dissociation constants we are enabled to plot the curves shown in figure 9, which reveal graphically the relationships of the various indicators in the series we shall discuss. This figure shows at a glance that an indicator of the simple type we have assumed has no appreciable dissociation and consequently exists in only one colored form at pH values begin-

ning about 2 points below the half transformation point, while at the same distance above this point the indicator is completely dissociated and exists only in its second form. Between these limits the color changes may be observed. The useful range of such an indicator is far less than 4 points of pH for optical reasons which will be discussed later.

The illustration (fig. 9) will show how in choosing a set of indicators it is advantageous to include a sufficient number, if reliable indicators can be found, so that their ranges overlap. It shows that each of the indicators, when considered to be of the simple type we have assumed, has an equal range. It also shows that the half transformation point of each indicator occurs nearer one end of the useful range, the useful range being indicated by the shaded part of the curve.

It is evident that if the actual color change of an indicator varied with pH in accordance with a curve such as those in figure 9, and if the true dissociation constant were accurately known, then the hydrogen ion concentration of a solution could be determined by finding the percentage transformation induced in the indicator. Indeed the dissociation constants of some few indicators have been determined with sufficient accuracy to permit the use of this method when the proper means of determining the color intensities are used. This will be discussed separately.

We have been assuming that the theory of indicators may be treated in the simple manner originally outlined by Ostwald (1891). In his theory it was assumed that the anion of an indicator acid, for instance, has a color different from that of the undissociated molecule. This assumption if unmodified does not harmonize with what is known. Researches in the phenomena of tautomerism have shown that when a change in color is observed in an indicator solution the change is associated with the formation of a new substance which is generally a molecular rearrangement or so-called "tautomer" of the old. If this color change is associated with the transformation of one substance into another, how is it that it seems to be controlled by the hydrogen ion concentration of the solution? As Steiglitz (1903) and others have pointed out, it is the state of these compounds, their existence in a dissociated or undissociated condition, which determines the stability of any one form.

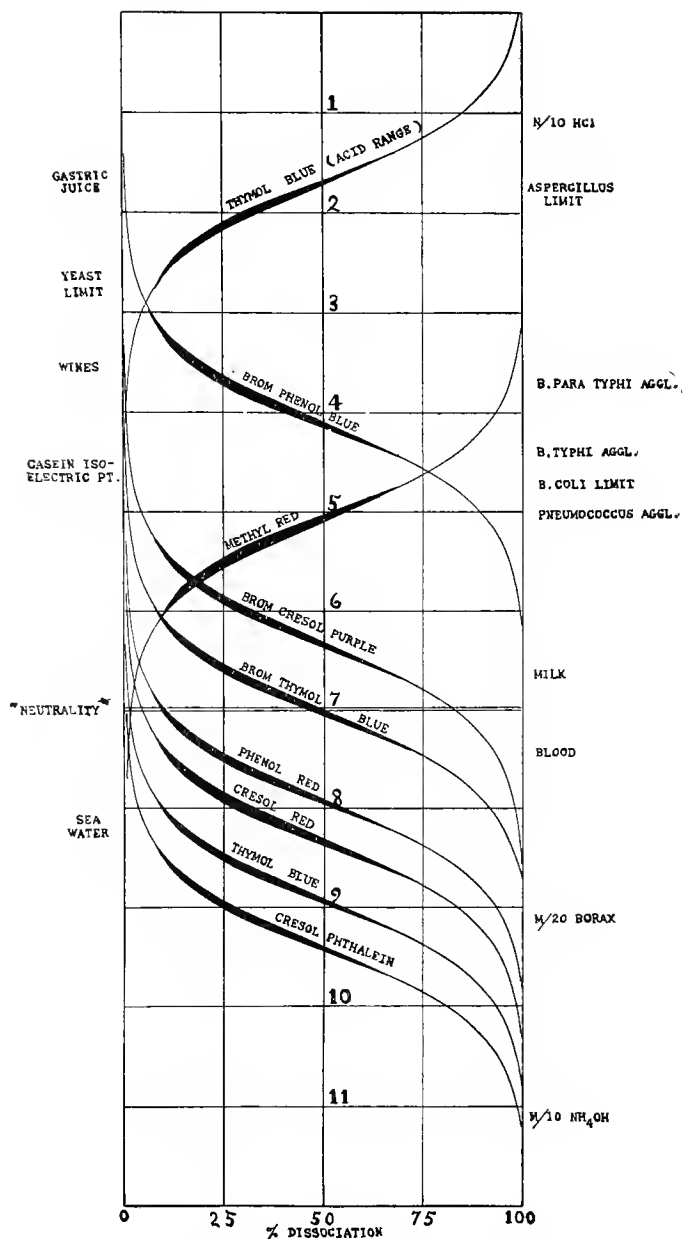


FIG. 9. INDICATOR CURVES AND SIGNIFICANT pH VALUES. SHADING INDICATES USEFUL RANGE

The method of dealing with the tautomeric relations of indicators is shown by the following quotation from Noyes (1910):

"We may derive a general expression (as has previously been done by Acrec, 1907) for the equilibrium-relations of any pair of tautomeric acids and their ions. The three fundamental equilibrium equations are as follows:

$$\frac{(\text{H}^+) (\text{In}'^-)}{(\text{HIn}')} = K_I'; \quad (12) \qquad \frac{(\text{H}^+) (\text{In}''^-)}{(\text{HIn}'')} = K_I; \quad (13)$$

$$\frac{(\text{HIn}'')}{(\text{HIn}')} = K_T; \quad (14)$$

Multiplying (13) by (14), adding (12) to the product, and substituting in the denominator for (HIn') its value $\frac{(\text{HIn}') + (\text{HIn}'')}{1 + K_T}$ given by (14), we get

$$\frac{(\text{H}^+) [(\text{In}'^-) + (\text{In}''^-)]}{(\text{HIn}') + (\text{HIn}'')} = \frac{K_I' + K_I'' K_T}{1 + K_T} = K_{IA} \quad (15)$$

If the indicator is a base existing as the two tautomeric substances $\text{In}'\text{OH}$ and $\text{In}''\text{OH}$, having ionization constants K_I' and K_I'' and a tautomer constant K_T defined by equations analogous to (12), (13) and (14), the general expression for the equilibrium between the ionized bases and their ions is:

$$\frac{(\text{OH}^-) [(\text{In}'^+) + (\text{In}''^+)]}{\text{In}'\text{OH} + \text{In}''\text{OH}} = \frac{K_I'^+ K_I'' K_T}{1 + K_T} = K_{IB} \quad (16)$$

In these expressions a single constant K_{IA} or K_{IB} has been introduced in place of the function of the three constants K_I' , K_I'' , and K_T The constants so calculated for a pair of tautomeric acids or bases can evidently be substituted for the ionization constant of an ordinary (non tautomeric) acid in any derived expression, provided the *sum* of the two ion concentrations and the sum of the two acid or base concentrations are quantities that are to be known or are to be calculated."

If then in equation (15) we substitute (In^-) for $[(\text{In}'^-) + (\text{In}''^-)]$ and (HIn) for $[(\text{HIn}') + (\text{HIn}'')]$ we have:

$$\frac{(\text{H}^+) (\text{In}^-)}{(\text{HIn})} = K_{IA} \quad (17)$$

Applying to (17) the derivation given on page 20

$$\alpha = \frac{K_{IA}}{K_{IA} + (\text{H}^+)}$$

From this we may plot the curves of figure 9. Such curves will then represent the color transformations when and only when (In^-) is substantially

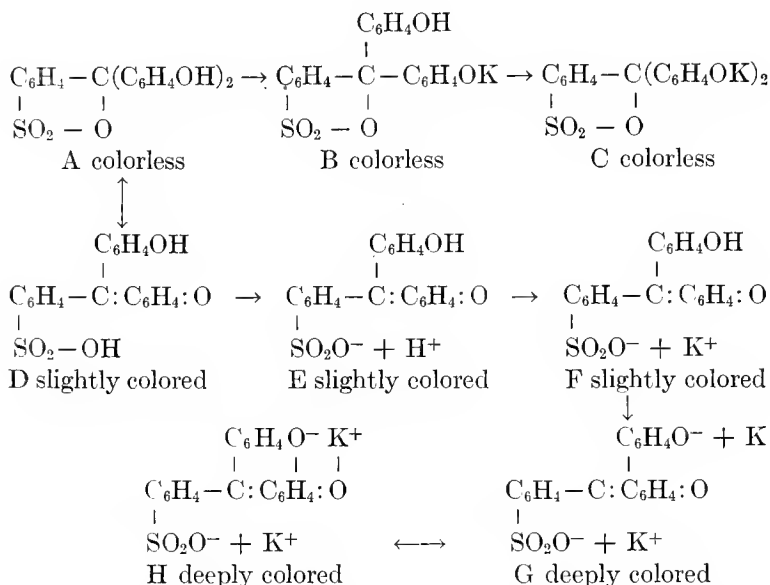
equal to (In'^-) or to (In''^-) , whichever tautomer is associated with the color. The most probable explanation of the fact that such curves do represent very closely the color transformations in certain instances is that K_T (see equation (14)) is so small that the dissociation brought about by salt formation leaves (In) dominant.

In other words it is, after all, the degree of dissociation, as determined by the hydrogen ion concentration, which determines which tautomer predominates. Therefore, consideration of the tautomeric equilibria only modifies the original Ostwald treatment to this extent; the true dissociation constant is a function of the several equilibrium and ionization constants involving the different tautomers and must be replaced by what Acree calls the "total affinity constant," or by what Noyes calls the "apparent dissociation constant," when it is desired to show directly how the color depends upon the hydrogen ion concentration.

Many indicators are poly-acidic or poly-basic and will not rigidly conform to the treatment for a simple mono-basic acid such as we have described. Phenolphthalein, for instance, as was shown by Acree (1908) and by Wegscheider (1908) must be considered as a poly-basic acid. The proper equations to apply in this case have been given by Acree (1907, 1908) and also by Wegscheider (1908, 1915). According to Acree and his students (Acree, 1908) (Acree and Slagle, 1909) the chief color change in phenolphthalein is associated with the presence of a quinone group and with the ionization of one of the phenol groups. In the sulfon phthalein series of indicators Acree and his students (White, 1915, and White and Acree, 1918) have found much the same sort of condition. In the sulfon phthalein series, however, certain unique properties described by Lubs and Acree (1916) make the series eminently suited for experimental demonstration of the seat of color change.

In the sulfon phthalein group of indicators we have to deal with dibasic acids; but as Acree has shown, the dissociation constant of the strong sulfonic acid group is so very much greater than that of the weak phenolic group, with which the principal color change is associated, that there is no serious interference. As shown in Chapter I we may, therefore, plot the curves as if we were dealing with a monobasic acid.

The structures of all the sulfon phthaleins are analogous to that of phenol sulfon phthalein (phenol red) whose various tautomers are given by Lubs and Acree (1916) in the following scheme:



The colorless lactoid A by reason of the strong tendency of the sulfonic acid group to ionize goes over into the quinoid structures illustrated in the second line which are slightly colored yellow. It is the transformation of F to G and H, the ionization of the phenolic group forming a quinone-phenolate structure which correlates with the intense red color of phenol sulfon phthalein (phenol red).

Just as the discovery of tautomeric rearrangements seemed at first to discredit the original Ostwald theory of color change, so it is now realized that the mere change in structure cannot of itself account for the light absorption upon which the color of a compound depends. Light is electromagnetic and if its absorption is to be accounted for in a *direct* manner we must search out the electromagnetic fields of force within the compound which take up the energy of particular wave lengths of light. It is in this direction that Baly (1915) believes the explanation of the color of dyes will be found. Although Baly has called attention to difficulties in the correla-

tion of color with tautomeric changes there seems to be no inherent reason why ionization, tautomerism, alteration in the fields of force within the compound and light absorption should not be correlated. The original Ostwald theory may yet prove to be essentially correct in that the electrical charges upon an ion must alter the fields of force to an extent which may or may not produce at the same time alteration in the "structure" of a compound and visible shifts in absorption spectra.

OPTICAL ASPECTS

While the color changes of indicators are correlated with molecular rearrangements controlled by hydrogen ion concentrations, it should not be forgotten that the phenomena observed are optical and that no theory of indicators can be considered complete enough for practical purposes which fails to recognize this. As ordinarily observed in laboratory vessels, the color observed is due to a somewhat complex set of phenomena. It is unfortunate that we have no adequate treatment of the subject which as the same time embraces electrolytic dissociation, tautomerism and the optical phenomena in a manner directly available in the practical application of indicators. The simultaneous treatment of these various aspects is necessary before we can feel quite sure of our ground when dealing with the discrepancies often observed in the comparison of colorimetric and electrometric measurements of biological fluids.

Let us first consider the range of an indicator as it is determined by the differentiating power of the eye. An approximate treatment of this is all that will be attempted.

Using equation (7) of page 20:

$$\log \frac{1}{[H^+]} = \text{pH} = \log \frac{1}{K} + \log \frac{\alpha}{(1 - \alpha)}$$

we find on differentiation that the rate of increase in α with increase of pH is:

$$\frac{d\alpha}{d(\text{pH})} = \alpha(1 - \alpha).$$

When

$$\frac{d^2\alpha}{d(\text{pH})^2} = 0, \alpha = \frac{1}{2}.$$

In other words the maximum rate of increase in dissociation is at the half transformation point. This fixes a reference point when indicators are to be employed in distinguishing differences in pH. The question now arises whether or not this is the central point of the optimum conditions for differentiation of pH values. It may be said at once that it is not because the eye has not only to detect differences but also to resolve these differences from the color already present. Experience shows that the point of maximum rate of increase in α is near one limit of the useful range and that this range lies on the side of lower dissociation. Thus, in the case of the one color indicator phenolphthalein, the useful zone of pH lies between about 8.4 and 9.8 instead of being centered at 9.7 which corresponds with the point of half transformation. In the case of a two color indicator such as phenol red the same reasoning holds, because the eye instinctively fixes upon the very dominant red. With other two color indicators the principle holds except when there is no very great difference in the command upon the attention by one or the other color.

The fixing of the lower limit of usefulness of a given indicator is not so simple as the fixing of the upper limit, because there is involved not only the percentage color but also the total indicator which may be brought into action. A dilute solution of phenolphthalein may appear quite colorless at pH 8.4 while a much stronger solution will show a distinct color which would permit distinguishing 8.2 from 8.4. The solubility of an indicator may alone determine where sufficient of the colored form can be present to permit detection of the first change of color with change in pH.

We ordinarily speak of color as if it were an entity. As a matter of fact the color exhibited by an indicator in solution is due to the selective absorption of certain wave lengths of the incident light. This results in the partial or complete blocking off of the light in one or more regions of the spectrum, as may be seen by the dark band or bands which appear when the solution is viewed through a spectrocope. The transmitted light instead of being of the continuous spectrum which blends to subjective white is made up of the unaffected wave lengths and of those whose intensities have been reduced to a greater or less extent. *The resultant subjective color* must be distinguished from the color associated with a definite region of the spectrum.

We come now to the consideration of a phenomenon which is undoubtedly exhibited with all indicators but which is generally not noticed except in special instances. In some of these instances it becomes of great importance and may lead to serious error unless recognized. The phenomenon we speak of is the dichromatism exhibited, for instance, by solutions of brom phenol blue. Solutions of this indicator appear blue when viewed in thin layers but red in deep layers. The explanation is as follows: The dominant absorption band of the alkaline solution is in the yellow and the green, so that the transmitted light is composed almost entirely of the red and blue. The incident light has an intensity which we may call I . After transmission through unit thickness of solution some of the light has been absorbed and the intensity becomes Ia , where a is a fraction—the transmission coefficient—which depends upon the nature of the absorbing medium and the wave length of the light. After traversing thickness ϵ the intensity becomes Ia^ϵ . Now the transmitted blue is $I_b a_b^\epsilon$ and the transmitted red $I_r a_r^\epsilon$. We do not happen to know what the actual values are, but, merely to illustrate the *principle*, let us assume first that the intensity of the incident blue is 100 and of the red 30 and that $a_b = 0.5$ and $a_r = 0.8$.

For $\epsilon = 1$, $I_b a_b^\epsilon = 50$ and $I_r a_r^\epsilon = 24$. Hence blue greater than red.

For $\epsilon = 10$, $I_b a_b^\epsilon = 0.01$ and $I_r a_r^\epsilon = 0.30$. Hence blue less than red.

This example indicates that the solution may appear blue when viewed through thin layers while it may appear red when viewed through thick layers.

If we change the relative intensities of the incident red and blue we can change the color of a given thickness of solution. If in the above example we reversed the intensities of the incident red and blue, then,

For $\epsilon = 1$, $I_b a_b^\epsilon = 15$ and $I_r a_r^\epsilon = 80$ or red greater than blue.

This is essentially what happens when we carry the solution from daylight, rich in blue, to the light of an electric carbon filament lamp, poor in blue. The solution which appears blue in daylight appears red in the electric light.

The practical importance of recognizing the nature of this phenomenon may be illustrated in the following way. Suppose we have a solution rich in suspended material such as bacterial cells, and that we wish to determine its pH value by using brom phenol blue. If we view such a solution in deep layers very little of the light incident at the bottom reaches the eye. A large proportion of the light which does reach the eye is that which has entered from the side, has been reflected by the suspended particles, and has traversed only a relatively thin section of the solution. In such a solution then, if it is of the proper pH, brom phenol blue will appear blue, while in a clear comparison solution of the same pH the indicator appears red or purple if the tube is viewed lengthwise. A comparison is therefore impossible under these conditions. If, however, we view the two solutions in relatively thin layers, as from the side of a test tube, they will appear more nearly comparable. There will still remain, however, a clearly recognizable difference in the quality of the color which serves as a warning that the two solutions are not being compared under proper conditions. We can obtain the proper conditions only when we eliminate from the source of light either the red or the blue, so that the phenomenon of dichromatism will not appear. Which had best be eliminated is a question which can not be answered properly until we have before us the necessary spectrometric measurements. Nevertheless the following observations made with a small hand spectroscope, and the deductions therefrom may prove to be illuminating.

The chief absorption bands of brom phenol blue solutions occur in the yellow-green range and in the blue. In alkaline solutions the band in the blue disappears while that in the yellow widens into the green. As the solution is made more acid the band in the blue appears, shutting off the transmitted blue, while that in the yellow-green contracts, permitting the passage of the green. Our light source then should be such that at least one of these changes may become apparent, and at the same time either the blue or red must be eliminated. The light of the mercury arc fulfills these conditions. It is relatively poor in red and it emits yellow, green and blue lines where the shifts in the absorption bands of brom phenol blue occur. Since the mercury arc is not generally available we have devised a light source to fulfill the

alternative conditions, namely, one which will permit observation of the contrasts due to the shift in the yellow-green band¹ and which at the same time is free from blue. Such a source is found in electric light from which the blue is screened by a translucent paper painted with an acid solution of phenol red. One disadvantage of such a screen is that the red transmitted through it is so dominant that it obscures the contrasts which are due to the shifting of the yellow-green absorption band. Nevertheless, such a screen has proved useful in pH determinations with brom phenol blue and particularly useful with brom cresol purple. In either case it is most useful in the more acid ranges covered by either of these indicators.

The device consists of an ordinary box of convenient size in which are mounted three or four large electric lights (e.g., 30 cp. carbon filaments). A piece of tin serves as reflector. The box may be lined with asbestos board. A piece of glass cut to fit the box is held in place on one side by the asbestos lining and on the other by a few tacks. This glass serves only to protect the screen and is not essential. The screen is made from translucent paper known to draughtsmen as "Economy" tracing paper. It is stretched across the open side of the box and painted with a solution consisting of 5 cc. of 0.6 per cent phenol red (stock solution of phenol sulfon phthalein) and 5 cc. of M/5 HK_2PO_4 (stock, standard phosphate solution). While the paper is wet it is stretched and pinned to the box with thumb tacks. This arrangement may be constructed in a very short time and will be found very helpful in many cases. It should be used in a dark room or, if such a room is not available, exterior light may be shut off with a photographer's black cloth.

While considering light sources we may call attention to the fact that all the sulfon phthalein indicators may be used in electric light, although brom thymol blue and thymol blue are not well adapted for use in light poor in blue. Doubtless a more thorough investigation of the absorption spectra of the sulfon phthalein indicators will make it possible to devise light sources which will materially increase their efficiency.

¹ This should not be confused with the changes in "subjective color." In the screened light no participation of transmitted green will be detected by the unaided eye.

So far as we have been able to detect with instruments at hand, the absorption spectra of all the indicators of the sulfon phthalein series are such that the appearance of dichromatism must be expected under certain conditions. It will be observed with phenol red in light relatively poor in red and rich in blue, for example, the light of a mercury arc; and with thymol blue in light relatively poor in blue and rich in red for example, ordinary electric light.

When the colorimeter is employed in the study of colored solutions the applicability of Beer's law is assumed. This may be expressed in the form, $\frac{L_1}{L_2} = \frac{C_2}{C_1}$ where C_1 and C_2 represent the concentrations of color in two solutions and L_1 and L_2 represent the depths of solution traveled by the light when a color match occurs. Applying this relation one is able to obtain the ratio of concentrations and therefrom the concentration in one solution if the concentration in the other be known. But as was shown above we have, in the case of two color indicators, different transmission coefficients for various regions of the spectrum. Consequently the depth of a solution cannot be altered as it is in the ordinary colorimeter without seriously altering the quality of the emergent light. This at once limits the usefulness of colorimeters in so far as their value depends upon alteration and measurement of the depth of solutions. That feature of some colorimeters which has to do with bringing the optical fields into juxtaposition remains most useful.

There have been two chief methods of dealing with the interfering effect of the natural color of solutions. The first method, used by Sørensen, consists in coloring the standard comparison solutions until their color matches that of the solution to be tested, and subsequently adding to each the indicator.

Sørensen's coloring solutions are the following:

- a. Bismarck brown (0.2 gram in 1 litre of water).
- b. Helianthin II (0.1 gram in 800 cc. alcohol, 200 cc. water).
- c. Tropaeolin O (0.2 gram in 1 litre of water).
- d. Tropaeolin OO (0.2 gram in 1 litre of water).
- e. Curcumein (0.2 gram in 600 cc. alcohol, 400 cc. water).
- f. Methyl violet (0.02 gram in 1 litre of water).
- g. Cotton blue (0.1 gram in 1 litre of water).

The second method was introduced by Walpole (1910). It consists in superimposing a tube of the colored solution over the standard comparison solution to which the indicator is added, and comparing this combination with the tested solution plus indicator superimposed upon a tube of clear water.

A somewhat crude but nevertheless helpful application of Walpole's principle may be made from a block of wood. Six deep holes just large enough to hold ordinary test tubes are bored parallel to one another in pairs. Adjacent pairs are placed as close to one another as can be done without breaking through the intervening walls. Perpendicular to these holes and running through each pair are bored *smaller* holes through which the test tubes may be viewed. The center pair of test tubes holds first the solution to be tested plus the indicator and second a water blank. At either side are placed the standards colored with the indicator and each backed by a sample of the solution under test. This is the so called "comparator" of Hurwitz, Meyer, and Ostenberg (1915). Before use it is well to paint the whole block and especially the holes a non-reflecting black.

This simple comparator is illustrated in figure 7.

One or another of the means described serves fairly well in overcoming the confusing influence of moderate color in solutions to be tested. In bacteriological work, however, a most serious difficulty is presented by the suspension of cells and precipitates.

If one views lengthwise a tube containing suspended particles, or even particles of colloid dimensions, much of the light incident at the bottom is absorbed or reflected before it reaches the eye, and, if the tube is not screened, some of the light which reaches the eye is that which has entered from the side and has been scattered. Consequently, a comparison with a clear standard is inadequate.

Sørensen (1909) has attempted to correct for this effect by the use of a finely divided precipitate suspended in the comparison solution. This he accomplishes by forming a precipitate of BaSO_4 through the addition of chemically equivalent quantities of BaCl_2 and Na_2SO_4 . Strictly speaking, this gives an imperfect imitation, but like the attempt to match color it does very well in many instances. The Walpole superposition method may be used with turbid solutions as well as with colored, as experience

with the device of Hurwitz, Meyer and Ostenberg has shown. In passing, attention should be called to the fact that the view of a turbid solution should be made through a relatively thin layer. When the comparison is made in test tubes, for instance, the view should be from the side.

There are some solutions, however, which are so dark or turbid that they cannot be handled with much precision by any of these methods. On the other hand a combination of these methods with moderate and judicious dilution, [as was indicated in Chapter I this may not seriously alter the pH of a solution] permits very good estimates with solutions which at first may appear impossible. Some of the deepest colored solutions permit reasonably good determinations and when sufficiently transparent permit the application of spectrometric devices. Turbidity on the other hand is sometimes unmanageable. Even in the case of milk where comparison with a standard is out of the question a two colored indicator presents a basis for judgment.

This brings us to a phase of the question the detailed analysis of which will not be attempted. It may simply be stated as a fact of experience that the color change of a two color indicator, presenting as it does change in intensities of what we may summarize as two colors, is a change in *quality* which is unmistakable within narrow limits. When there is added to this that brilliancy which is characteristic of the sulfon phthalein indicators the subjective aspect of indicator work is taken care of in a way that may surprise one.

The spectrophotometer and allied instruments which have served in many of the investigations of indicators have not yet been brought within the range of ordinary colorimetric procedure for the determination of pH. Where there occurs a great change in the absorption bands as at the endpoint of a titration the hand spectroscope may be applied but it is doubtful if such an instrument is of much value for slight differences of virage. For the possibilities which remain for development in this field the reader is referred to the special literature.

This sketch of some of the principal aspects of indicator theory would be incomplete were attention not called to the value of indicators in demonstrating to students many of the important relations of acids and bases.

REFERENCES

- In the Theory and Use of Indicators, by E. B. R. Prideaux, published in London, 1917, will be found a résumé of important aspects of indicator theory and numerous references.
- See also recent papers by Acree and associates in the Journal of the American Chemical Society on Sulfon phthaleins.

CHAPTER IV

CHOICE OF INDICATORS

From the enormous number of colored compounds found in nature and among the products of the laboratory many may be chosen for their special value as acidimetric-alkalimetric indicators. Among those of plant origin litmus and alizarine are the more familiar. One indicator of animal origin, cochineal, an extract of an insect, was formerly used to some extent. Walpole's (1913) treatment of litmus, Walbum's (1913) study of the coloring matter of the red cabbage and some of the more recent work done in connection with the cell penetration of acids has given us a little data on properties of plant and animal pigments which are applicable to hydrogen ion determinations. But for the most part indicators of natural origin have been neglected for the study of synthetic compounds.

Litmus has played so important a rôle in acidimetry that it is worthy of brief, special mention.

Litmus is obtained by the oxidation in the presence of ammonia of the orcin contained in lichens, generally of the species *Roccella* and *Lecanora*. The material which comes upon the market is frequently heavily laden with salts. The coloring matter is a complex (Glazer, 1901) the composition of which will vary with the numerous methods of extraction and with the source. The azolitmin of commerce is also of uncertain composition (Scheitz, 1910) but is considered to be the chief indicator present in litmus. The following method of preparing a sensitive litmus solution is taken from Morse (1905).

The crushed commercial litmus is repeatedly extracted with fresh quantities of 85 per cent alcohol for the purpose of removing a violet coloring matter which is colored by acids but not made blue by alkalis. The residue, consisting mainly of calcium carbonate, carbonates of the alkalis and the material to be isolated, is washed with more hot alcohol upon a filter and then digested for several hours with cold distilled water. The filtered aqueous extract has a pure blue color and contains an excess of alkali, a part of which is in the form of carbonate and a part in combination with litmus. To remove the alkaline reaction the solution is heated to the boil-

ing point and cautiously treated with very dilute sulfuric acid until it becomes very distinctly and permanently red. Boil till all CO_2 is dispelled. Treat with a dilute solution of barium hydroxide until the color changes to a violet. Filter, evaporate to a small volume and precipitate the litmus with strong alcohol. Wash with alcohol and dry.

Dr. Rupp of this laboratory prefers to make a final washing with water which removes much of the salts at the expense of some dye.

Synthetic indicators have for the most part displaced those of natural origin until litmus and alizarine, turmeric and cochineal are becoming more and more unfamiliar in the chemical laboratory. Indeed Bjerrum (1914) states that the two synthetic indicators, methyl red and phenolphthalein, particularly because of the zones of hydrogen ion concentration within which they change color, are sufficient for most titrimetric purposes.

But the two indicators mentioned above cover but a very limited range of hydrogen ion concentration so that they are insufficient for the purpose we now have under consideration. A survey of indicators suitable for hydrogen ion determinations was opened in Nernst's laboratory in 1904 by Salesky. This survey was extended in the same year by Friedenthal, by Fels and by Salm and the results were summarized in Salm's famous table (cf. *Z. physik. Chem.*, **57**).

Then came the classic work of Sørensen of the Carlsberg laboratory in Copenhagen. The array of available indicators had become so large as to be burdensome. Sørensen in an extensive investigation of the correspondence between colorimetric and electrometric determinations of hydrogen ion concentrations revealed discrepancies which were attributed mainly to the influence of protein and salts. He chose those indicators which were relatively free from the so-called protein and salt errors, constructed solutions of known and reproducible hydrogen ion concentration and thus furnished the biochemist with selected tools of beautiful simplicity. It is well to emphasize the labor of elimination which Sørensen performed because without it we might still have been consulting such tables as the ponderous one published by Thiel (1911) and be bewildered by the very extensive array.

Sørensen's final selection together with the pH range of each indicator is given at the end of this chapter.

After giving his table of selected indicators Sørensen remarks:

Not all these indicators furnish equally well defined "virages" and above all they are not of equal applicability under all circumstances. In the choice of an indicator from among those which we have been led to recommend it is necessary to use judicious care and especially to take into consideration the following facts:

a. The indicators of the methyl violet group (nos. 1 and 2) (see table 4) are especially sensitive to the action of neutral salts; furthermore the intensity of color changes on standing and the change is the more rapid the more acid the medium.

b. The basic indicators (nos. 3, 6, 9, 11, 14) are soluble in toluene and in chloroform. The first four separate partially on prolonged standing of the experimental solution.

c. In the presence of high percentages of natural proteins most of the indicators are useless although certain of them are still serviceable; nos. 1, 2, 13, 16, 17, 18.

d. In the presence of protein decomposition products even in considerable proportions the entire series of indicators may render real service. Yet even in these conditions some of the acid azo indicators may give notable errors (nos. 4, 5, 7, 8, 10) in which case one should resort to the corresponding basic indicators.

e. When only small percentages of protein or their decomposition products are concerned the acid azo indicators are more often preferable to the basic for they are not influenced by toluene or chloroform and do not separate from solution on standing.

f. In all doubtful cases—for example in the colorimetric measurement of solutions whose manner of reaction with the indicator is not known, the electrometric measurement as a standard method should be used. Then the worth of the indicator will be determined by electrometric measurement with colorimetric comparison.

Sørensen's work, coupled as it was with a most important contribution to enzyme chemistry gave great impetus to the use of indicators in biochemistry. His selection of indicators was therefore soon enlarged by additions of new indicators which fulfilled the criteria of reliability which he had laid down. Alpha naphthol phthalein, a compound first synthesized by Grabowsik (1871), was shown by Sørensen and Palitzsch (1910) to have a pH range of pH 7–9 and was found useful in biological fluids. Methyl red (Rupp and Loose, 1908) was given its very useful place by the investigations of Palitzsch (1911). Henderson and Forbes (1910) introduced 2–5 di nitro hydroquinone as an indicator possessing several steps of color change and therefore useful over a wide range of pH. Walpole (1914) called attention to several indi-

cators of potential value. Hottinger (1914) recommended "lacmosol," a constituent of lacmoid. These and numerous other indicators such as Dox's (1915) phenol quinolinein, Scatchard and Bogert's (1916) di nitro benzoylene urea, and Rupp's (1915) syntheses in the methyl red series, present a wealth of material but little of which has been thoroughly worked over.

In 1915 Levy, Rowntree and Marriot, without applying the tests of reliability which Sørensen had employed, used phenol sulphon phthalein in determining the pH of the dializate of blood. This compound first synthesized in Remsen's laboratory by Sohon (1898) has received considerable attention from Acree and his co-workers because it furnishes excellent material for the quinone-phenolate theory of indicators. To further such studies Acree and White had synthesized new derivatives of phenol sulphon phthalein at the time when the work of Levy, Rowntree and Marriot attracted the attention of Lubs and Clark. These authors were looking for more brilliant indicators for use in bacterial culture media and were attracted by the well known brilliance of phenol sulphon phthalein. Through the courtesy of Professor Acree some of the derivatives which White had prepared were obtained. New homologues were synthesized by Lubs. The applicability of these and numerous other indicators in the determination of the pH values of biological fluids was then studied.

In the sulfon phthalein series the following were studied:

Phenol sulfon phthalein, Sohon (1898).

Tetra nitro phenol sulfon phthalein, White and Acree (1915).

Phenol nitro sulfon phthalein, Lubs and Clark (1915).

Tetra bromo phenol sulfon phthalein, White and Acree (1915).

Tetra chloro phenol sulfon phthalein, Lubs and Clark.

Ortho cresol sulfon phthalein, Sohon (1898).

Di bromo ortho cresol sulfon phthalein, Sohon (1898).

Thymol sulfon phthalein, Lubs and Clark (1915).

Thymol nitro sulfon phthalein, Lubs and Clark.

Di bromo thymol sulfon phthalein, Lubs and Clark (1915).

α -naphthol sulfon phthalein, Lubs and Clark (1915).

Carvacrol sulfon phthalein, Lubs and Clark.

Orcinol sulfon phthalein, Gilpin (1894).

In the course of this work there were studied:

o-carboxy benzene azo mono methyl aniline, Sive and Jones (1915).

o-carboxy benzene azo di methyl aniline, Rupp and Loose (1908).

o-carboxy benzene azo mono ethyl aniline, Lubs and Clark (1915).

o-carboxy benzene azo di ethyl aniline, Lubs and Clark (1915).

o-carboxy benzene azo mono propyl aniline, Lubs and Clark (1915).

o-carboxy benzene azo di propyl aniline, Lubs and Clark (1915).

o-carboxy benzene azo (?) amyl aniline, Lubs and Clark (1915).

o-carboxy benzene azo di methyl α naphthyl amine, Howard and Pope (1911).

o-carboxy benzene azo α naphthyl amine, Howard and Pope (1911).

o-carboxy benzene azo di phenyl amine, Howard and Pope (1911).

Meta carboxy benzene azo di methyl aniline, Lubs and Clark.

The mono alkyl homologues of methyl red were found to be much less brilliant than the di alkyl compounds and were therefore rejected. For the same reason or because of large protein errors we rejected the other compounds with the exception of di ethyl and di propyl red. Of these we retained di propyl red because it is very useful in solutions of a little lower hydrogen ion concentration than those which may be studied with methyl red.

Propyl red is, however, not included in table 3 because it precipitates too easily from buffer solutions to be of general usefulness. It is also difficult to obtain on the market.

As the result of an extensive series of comparisons between colorimetric and electrometric measurements, made for the most part upon solutions of interest to bacteriologists, Clark and Lubs (1917) suggested the series of indicators given in table 3. This series is made up for the most part of the brilliant and more reliable sulfon phthaleins but contains the still indispensable but not very stable methyl red.

In the course of their investigations these authors resurrected ortho cresol phthalein (Baeyer and Freude, 1880), found it quite as reliable as phenolphthalein and more brilliant with a color better adapted to titrations in artificial light.

In table 3 will be found the final selection of Clark and Lubs with the common names which they suggested for laboratory parlance, the concentration of indicator convenient for use, a rough indication of the nature of the color, and the useful pH range.

TABLE 3
Clark and Lubs' list of indicators

CHEMICAL NAME	COMMON NAME	CONCENTRATION	COLOR CHANGE	RANGE pH
		<i>per cent</i>		
Thymol sulfon phthalein (acid range).....	Thymol blue (see below)	0.04	Red-yellow	1.2-2.8
Tetra bromo phenol sulfon phthalein...	Brom phenol blue	0.04	Yellow-blue	3.0-4.6
Ortho carboxy benzene azo di methyl aniline.....	Methyl red	0.02	Red-yellow	4.4-6.0
Di bromo ortho cresol sulfon phthalein.....	Brom cresol purple	0.04	Yellow-purple	5.2-6.8
Di bromo thymol sulfon phthalein....	Brom thymol blue	0.04	Yellow-blue	6.0-7.6
Phenol sulfon phthalein.....	Phenol red	0.02	Yellow-red	6.8-8.4
Ortho cresol sulfon phthalein.....	Cresol red	0.02	Yellow-red	7.2-8.8
Thymol sulfon phthalein.....	Thymol blue	0.04	Yellow-blue	8.0-9.6
Ortho cresol phthalein.....	Cresol phthalein..	0.02	Colorless-red	8.2-9.8

With the improved method for the preparation of the sulfon phthalein indicators described by Lubs and Clark (1915) they may easily be made from materials readily obtained. The indicators can also now be purchased in this country from chemical supply houses.

The indicators recommended by Clark and Lubs are marketed both in the form of a dry powder and in stock solutions. In cases

where the acidity of the free acid dye in the indicator solution does not interfere with accuracy and when alcohol is not objectionable the alcoholic solutions of the dyes may be used. Clark and Lubs prefer to use aqueous solutions of the alkali salts in concentrations which may be conveniently kept as stock solutions. These are diluted for the test solutions used in the dropping bottles.

For the preparation of these stock solutions one decigram (0.1 gram) of the dry powder is ground in an agate mortar with the following quantities of N/20 NaOH. When solution is complete dilute to 25 cc.

MOLECULAR WEIGHT	INDICATOR	N/20 NaOH PER DECIGRAM
		cc.
354.17	Phenol red	5.7
669.82	Brom phenol blue	3.0
382.17	Cresol red	5.3
540.01	Brom cresol purple*	3.7
466.30	Thymol blue	4.3
624.12	Brom thymol blue	3.2
269.12	Methyl red	7.4

If there be no particular reason to maintain exact equivalents it may be found easier to dissolve the dyes in 1.1 equivalents of alkali instead of one equivalent as indicated above.

When made up to 25 cc. as noted above there is obtained in each case a 0.4 per cent solution of the original dye itself. For tests they should be diluted further. For use in testing 10 cc. of a solution with five drops of indicator solution good concentrations are 0.04 per cent for thymol blue, brom thymol blue, brom phenol blue, and brom cresol purple, and 0.02 per cent for cresol red, phenol red and methyl red.

Methyl red may be more conveniently prepared for the tests by dissolving one decigram in 300 cc. alcohol and diluting to 500 cc. with distilled water.

Ortho cresol phthalein and phenolphthalein are used in a 0.02 per cent solution in 95 per cent alcohol.

* Poor grades of this indicator decompose when first taken up in alkali. In such a case use the alcoholic solution.

TABLE 4

Sørensen's selected indicators and their pH ranges

INDICATOR	pH RANGE
1. Methyl violet.....	0.1-3.2
2. Mauveine.....	-0.1-2.9
3. Diphenylamino-azo-benzene.....	1.2-2.1
4. Diphenylamino-azo-parabenzene sulfonic acid (Tropeolin OO).....	1.4-2.6
5. Diphenylamino-azo-metabenzene sulfonic acid.....	1.2-2.3
6. Benzylanilino-azo-benzene.....	2.3-3.3
7. Benzylanilino-azo-parabenzene sulfonic acid.....	1.9-3.3
8. Metachloro diethyl anilino-azo-parabenzene sulfonic acid.....	2.6-4.0
9. Dimethylanilino-azo-benzene.....	2.9-4.0
10. Methyl orange.....	3.1-4.4
11. α -naphthylamino-azo-benzene.....	3.7-5.0
12. α -naphthylamino-azo-parabenzene sulfonic acid.....	3.5-5.7
13. p-nitrophenol.....	5.0-7.0
14. Neutral red.....	6.8-8.0
15. Rosolic acid.....	6.9-8.0
16. Tropeolin OOO.....	7.6-8.9
17. Phenolphthalein.....	8.3-10.0
18. Thymolphthalein.....	9.3-10.5
19. p-nitrobenzene-azo-salicylic acid (Alizarine Yellow G)...	10.1-12.1
20. Resorcine-azo-parabenzenesulfonic acid (Tropeolin O)...	11.1-12.7

TABLE 5

Miscellaneous indicators

INDICATOR	pH RANGE
Alizarine.....	{ 5.5-6.8 (Sørensen) 10.1-12.1
Alizarine blue S.....	11-13 (Prideaux)
Azolitmin.....	4.5-8.3 (Sørensen)
α -naphthol benzoin.....	9-12
α -naphthol phthalein.....	7.2-8.6 (Sørensen and Palitzsch)
Cochineal.....	4.8-6.2 (Sørensen)
Congo red.....	3-5 (Prideaux).....
Cyanin.....	7-8 (Prideaux)
Dinitrobenzoylene urea.....	6-8 (Bogert and Scatchard)
Lacmoid.....	4.4-6.2 (Sørensen)

TABLE 5—*Continued*

INDICATOR	pH RANGE
Lacmosol.....	4.4–5.5 (Hottinger)
Litmus, see azolitmin	
Poirrier's blue.....	11–13 (Prideaux)
Propyl red.....	4.8–6.4 (Lubs and Clark)
Red cabbage extract.....	2.4–4.5 (Walbum)
2–5 dinitro hydroquinone.....	3–9 (Henderson and Forbes)

CHAPTER V

STANDARD BUFFER SOLUTIONS FOR COLORIMETRIC COMPARISON

The standard solutions used in the colorimetric method of determining hydrogen ion concentrations are buffer solutions with such well defined compositions that they can be accurately reproduced, and with pH values accurately defined by hydrogen electrode measurements. They generally consist of mixtures of some acid and its alkali salt. Several such mixtures have been carefully studied. An excellent set has been described by Sørensen (1912). This set may be supplemented by the acetic acid—sodium acetate mixtures, most careful measurements of which have been made by Walpole (1914), and by Palitzsch's (1915) excellent boric acid-borax mixtures.

Clark and Lubs (1916) have designed a set of standards which they believe are somewhat more convenient in preparation than are the Sørensen standards. Their set is composed of the following mixtures:

Potassium chlorid + HCl
Acid potassium phthalate + HCl
Acid potassium phthalate + NaOH
Acid potassium phosphate + NaOH
Boric acid, KCl + NaOH

For a discussion of these mixtures, the methods used in determining their pH values, and the potential measurements we refer the reader to the original paper (*Journal of Biological Chemistry*, 1916, **25**, no. 3, p. 479). We may proceed at once to describe the details of preparation.

The various mixtures are made up from the following stock solutions: M/5 potassium chlorid (KCl), M/5 acid potassium phosphate (KH_2PO_4), M/5 acid potassium phthalate ($\text{KHC}_8\text{H}_4\text{O}_4$), M/5 boric acid with M/5 potassium chlorid (H_3BO_3 , KCl), M/5 sodium hydroxid (NaOH), and M/5 hydrochlorid acid (HCl). Although the subsequent mixtures are diluted to M/20 the above concentrations of the stock solutions are convenient for several reasons.

The water used in the crystallization of the salts and in the preparation of the stock solutions and mixtures should be redistilled. So-called "conductivity water," which is distilled first from acid chromate solution and again from barium hydroxid, is recommended, but it is not necessary.

M/5 potassium chlorid solution. (This solution will not be necessary except in the preparation of the most acid series of mixtures.) The salt should be recrystallized three or four times and dried in an oven at about 120°C. for two days. The fifth molecular solution contains 14.912 grams in 1 liter.

M/5 acid potassium phthalate solution. Acid potassium phthalate may be prepared by the method of Dodge (1915) modified as follows. Make up a concentrated potassium hydroxid solution by dissolving about 60 grams of a high grade sample in about 400 cc. of water. To this add 50 grams of the commercial *resublimed* anhydrid of ortho phthalic acid. Test a cool portion of the solution with phenol phthalein. If the solution is still alkaline, add more phthalic anhydrid; if acid, add more KOH. When roughly adjusted to a slight pink with phenol phthalein¹ add as much more phthalic anhydrid as the solution contains and heat till all is dissolved. Filter while hot, and allow the crystallization to take place slowly. The crystals should be drained with suction and recrystallized at least twice from distilled water.² Dry the salt at 110°–115°C. to constant weight.

A fifth molecular solution contains 40.828 grams of the salt in 1 liter of the solution.

M/5 acid potassium phosphate solution. A high grade commercial sample of the salt is recrystallized at least three times from distilled water and dried to constant weight at 110°–115°C. A fifth molecular solution should contain in 1 liter 27.232 grams. The solution should be distinctly red with methyl red and distinctly blue with brom phenol blue.

¹ Use a diluted portion for the final test.

² While the present price of phthalic acid continues it will be well to recover the phthalic acid from the mother liquors by acidifying these. The recovered phthalic acid may be easily and economically purified by several recrystallizations.

Samples of phthalic anhydrid which are now found on the market are frequently grossly impure. With some samples ten recrystallizations are necessary. Hence it is economy to purchase only the highest grades.

M/5 boric acid M/5 potassium chlorid. Boric acid should be recrystallized several times from distilled water. It should be air dried³ in thin layers between filter paper and the constancy of weight established by drying small samples in thin layers in a desiccator over CaCl_2 . Purification of KCl has already been noted. It is added to the boric acid solution to bring the salt concentration in the borate mixtures to a point comparable with that of the phosphate mixtures so that colorimetric checks may be obtained with the two series where they overlap. One liter of the solution should contain 12.4048⁴ grams of boric acid and 14.912 grams of potassium chlorid.

M/5 sodium hydroxid solution. This solution is the most difficult to prepare, since it should be as free as possible from carbonate. A solution of sufficient purity for the present purposes may be prepared from a high grade sample of the hydroxid in the following manner. Dissolve 100 grams NaOH in 100 cc. distilled water in a Jena or Pyrex glass Erlenmeyer flask. Cover the mouth of the flask with tin foil and allow the solution to stand over night till the carbonate has settled. Then prepare a filter as follows. Cut a "hardened" filter paper to fit a Buchner funnel. Treat it with warm, strong [1:1] NaOH solution. After a few minutes decant the sodium hydroxid and wash the paper first with absolute alcohol, then with dilute alcohol, and finally with large quantities of distilled water. Place the paper on the Buchner funnel and apply gentle suction until the greater part of the water has evaporated; but do not dry so that the paper curls. Now pour the concentrated alkali upon the middle of the paper, spread it with a glass rod making sure that the paper, under gentle suction, adheres well to the funnel, and draw the solution through with suction. The clear filtrate is now diluted quickly, after rough calculation, to a solution somewhat more concentrated than N/1. Withdraw 10 cc. of this dilution and standardize roughly with an acid solution of known strength, or with a sample

³ Boric acid begins to lose "water of constitution" above 50°C.

⁴ This weight was used on the assumption that the atomic weight of boron is 11.0. The atomic weight has since been revised and appears as 10.9 in the 1920 table.

Because the solutions were standardized with the above weight of boric acid this weight should be used.

of acid potassium phthalate. From this approximate standardization calculate the dilution required to furnish an M/5 solution. Make the required dilution with the least possible exposure, and pour the solution into a *paraffined*⁵ bottle to which a calibrated 50 cc. burette and soda-lime guard tubes have been attached. The solution should now be most carefully standardized. One of the simplest methods of doing this, and one which should always be used in this instance, is the method of Dodge (1915) in which use is made of the acid potassium phthalate purified as already described. Weigh out accurately on a chemical balance with standardized weights several portions of the salt of about 1.6 grams each. Dissolve in about 20 cc. distilled water and add 4 drops phenol phthalein. Pass a stream of CO₂-free air through the solution and titrate with the alkali till a faint but distinct and permanent pink is developed. It is preferable to use a factor with the solution rather than attempt adjustment to an exact M/5 solution.

M/5 hydrochloric acid solution. Dilute a high grade of hydrochloric acid solution to about 20 per cent and distill. Dilute the distillate to approximately M/5 and standardize with the sodium hydroxid solution previously described. If convenient, it is well to standardize this solution carefully by the silver chlorid method and check with the standardized alkali.

The only solution which it is absolutely necessary to protect from the CO₂ of the atmosphere is the sodium hydroxid solution. Therefore all but this solution may be stored in ordinary bottles of resistant glass. The salt solutions, if adjusted to exactly M/5, may be measured from clean calibrated pipettes.

These constitute the stock solutions from which the mixtures are prepared. The general relationships of these mixtures to their pH values are shown in figure 10. In this figure pH values are plotted as ordinates against X cc. of acid or alkali as abscissas. It will be found convenient to plot this figure from table 6 with

⁵ The author finds that thick coats of paraffine are more satisfactory than the thin coats sometimes recommended. Thoroughly clean and *dry* the bottle, warm it and then pour in the melted paraffine. Roll gently to make an even coat and just before solidification occurs stand the bottle upright to allow excess paraffine to drain to the bottom and there form a very substantial layer.

greatly enlarged scale so that it may be used as is Sørensen's chart (1909). The compositions of the mixtures at even intervals of 0.2 pH are given in table 6.

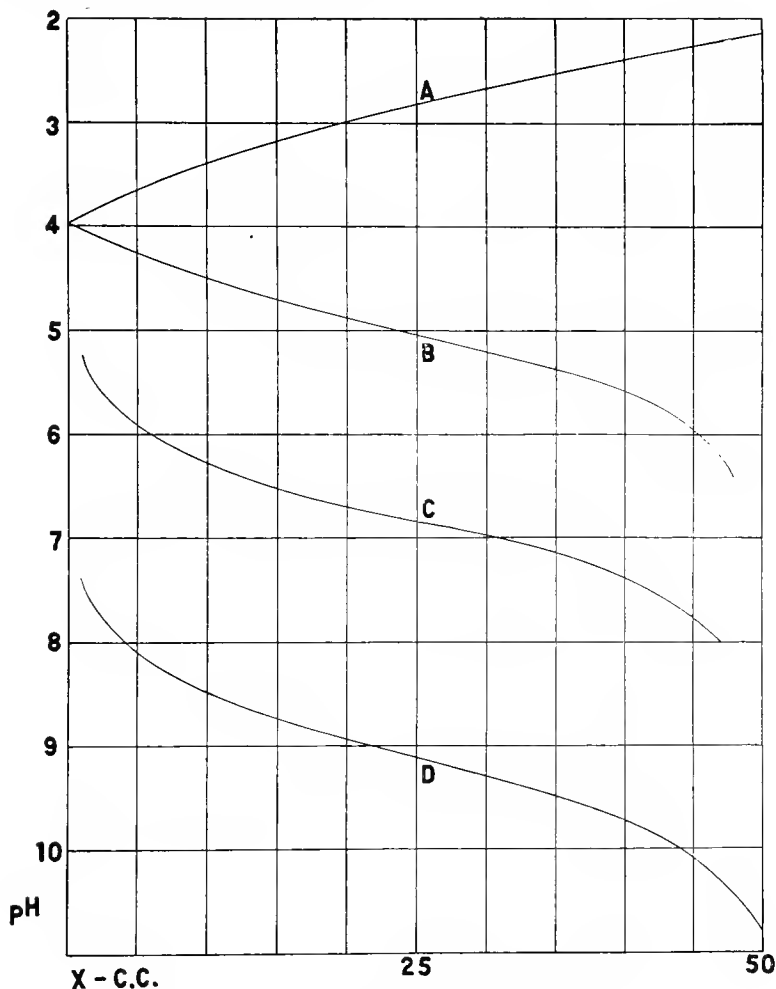


FIG. 10. CLARK AND LUBS' STANDARD MIXTURES

- A. 50 cc. 0.2M KHPthalate + X cc. 0.2M HCl. Diluted to 200 cc.
 B. 50 cc. 0.2M KHPthalate + X cc. 0.2M NaOH. Diluted to 200 cc.
 C. 50 cc. 0.2M KH_2PO_4 + X cc. 0.2M NaOH. Diluted to 200 cc.
 D. 50 cc. 0.2M H_3BO_3 , 0.2M KCl + X cc. 0.2M NaOH. Diluted to 200 cc.

In any measurement the apportionment of scale divisions should accord with the precision. Scale divisions should not be so coarse that interpolations tax the judgment nor so fine as to be ridiculous. What scale divisions are best in the method under discussion it is difficult to decide, since the precision which may be attained depends somewhat upon the ability of the individual eye, and upon the material examined, as well as upon the means and the judgment used in overcoming certain difficulties which we shall mention later. Certain general considerations have led us to believe that for most work estimation of pH values to the nearest 0.1 division is sufficiently precise, and that this precision can be obtained when the composition of the medium permits if the comparison standards differ by increments of 0.2 pH. Sørensen (1909) has arranged the standard solutions to differ by even parts of the components, a system which furnishes uneven increments in pH. Michaelis (1910), on the other hand, makes his standards vary by about 0.3 pH so that the corresponding hydrogen ion concentrations are approximately doubled at each step. Our experience has convinced us of the advantage of the 0.2 pH increments we are recommending.

We have found it convenient to prepare 200 cc. of each of the mixtures and to preserve them in bottles each of which has its own 10 cc. pipette thrust through the stopper. It takes but little more time to prepare 200 cc. than it does to prepare a 10 cc. portion, and if the larger volume is prepared there will not only be a sufficient quantity for a day's work but there will be some on hand for the occasional test.

Unless electrometric measurements can be used as control, we urge the most scrupulous care in the preparation and preservation of the standards. We have specified several recrystallizations of the salts used because no commercial samples which we have yet encountered are reliable.

It is important to check the consistency of any particular set of these mixtures by comparing "5.8" and "6.2 phthalate" with "5.8" and "6.2 phosphate" using brom cresol purple. Also "7.8" and "8.0 phosphate" should be compared with the corresponding borates using cresol red.

TABLE 6

Composition of mixtures giving pH values at 20°C. at intervals of 0.2

KCl-HCl mixtures*

pH			
1.2	50 cc. M/5 KCl	64.5 cc. M/5 HCl	Dilute to 200 cc.
1.4	50 cc. M/5 KCl	41.5 cc. M/5 HCl	Dilute to 200 cc.
1.6	50 cc. M/5 KCl	26.3 cc. M/5 HCl	Dilute to 200 cc.
1.8	50 cc. M/5 KCl	16.6 cc. M/5 HCl	Dilute to 200 cc.
2.0	50 cc. M/5 KCl	10.6 cc. M/5 HCl	Dilute to 200 cc.
2.2	50 cc. M/5 KCl	6.7 cc. M/5 HCl	Dilute to 200 cc.

* The pH values of these mixtures are given by Clark and Lubs (1916) as *preliminary* measurements.

Phthalate-HCl mixtures

2.2	50 cc. M/5 KHPhtalate	46.70 cc. M/5 HCl	Dilute to 200 cc.
2.4	50 cc. M/5 KHPhtalate	39.60 cc. M/5 HCl	Dilute to 200 cc.
2.6	50 cc. M/5 KHPhtalate	32.95 cc. M/5 HCl	Dilute to 200 cc.
2.8	50 cc. M/5 KHPhtalate	26.42 cc. M/5 HCl	Dilute to 200 cc.
3.0	50 cc. M/5 KHPhtalate	20.32 cc. M/5 HCl	Dilute to 200 cc.
3.2	50 cc. M/5 KHPhtalate	14.70 cc. M/5 HCl	Dilute to 200 cc.
3.4	50 cc. M/5 KHPhtalate	9.90 cc. M/5 HCl	Dilute to 200 cc.
3.6	50 cc. M/5 KHPhtalate	5.97 cc. M/5 HCl	Dilute to 200 cc.
3.8	50 cc. M/5 KHPhtalate	2.63 cc. M/5 HCl	Dilute to 200 cc.

Phthalate-NaOH mixtures

4.0	50 cc. M/5 KHPhtalate	0.40 cc. M/5 NaOH	Dilute to 200 cc.
4.2	50 cc. M/5 KHPhtalate	3.70 cc. M/5 NaOH	Dilute to 200 cc.
4.4	50 cc. M/5 KHPhtalate	7.50 cc. M/5 NaOH	Dilute to 200 cc.
4.6	50 cc. M/5 KHPhtalate	12.15 cc. M/5 NaOH	Dilute to 200 cc.
4.8	50 cc. M/5 KHPhtalate	17.70 cc. M/5 NaOH	Dilute to 200 cc.
5.0	50 cc. M/5 KHPhtalate	23.85 cc. M/5 NaOH	Dilute to 200 cc.
5.2	50 cc. M/5 KHPhtalate	29.95 cc. M/5 NaOH	Dilute to 200 cc.
5.4	50 cc. M/5 KHPhtalate	35.45 cc. M/5 NaOH	Dilute to 200 cc.
5.6	50 cc. M/5 KHPhtalate	39.85 cc. M/5 NaOH	Dilute to 200 cc.
5.8	50 cc. M/5 KHPhtalate	43.00 cc. M/5 NaOH	Dilute to 200 cc.
6.0	50 cc. M/5 KHPhtalate	45.45 cc. M/5 NaOH	Dilute to 200 cc.
6.2	50 cc. M/5 KHPhtalate	47.00 cc. M/5 NaOH	Dilute to 200 cc.

KH_2PO_4 -NaOH mixtures

5.8	50 cc. M/5 KH_2PO_4	3.72 cc. M/5 NaOH	Dilute to 200 cc.
6.0	50 cc. M/5 KH_2PO_4	5.70 cc. M/5 NaOH	Dilute to 200 cc.
6.2	50 cc. M/5 KH_2PO_4	8.60 cc. M/5 NaOH	Dilute to 200 cc.
6.4	50 cc. M/5 KH_2PO_4	12.60 cc. M/5 NaOH	Dilute to 200 cc.
6.6	50 cc. M/5 KH_2PO_4	17.80 cc. M/5 NaOH	Dilute to 200 cc.
6.8	50 cc. M/5 KH_2PO_4	23.65 cc. M/5 NaOH	Dilute to 200 cc.
7.0	50 cc. M/5 KH_2PO_4	29.63 cc. M/5 NaOH	Dilute to 200 cc.
7.2	50 cc. M/5 KH_2PO_4	35.00 cc. M/5 NaOH	Dilute to 200 cc.
7.4	50 cc. M/5 KH_2PO_4	39.50 cc. M/5 NaOH	Dilute to 200 cc.
7.6	50 cc. M/5 KH_2PO_4	42.80 cc. M/5 NaOH	Dilute to 200 cc.
7.8	50 cc. M/5 KH_2PO_4	45.20 cc. M/5 NaOH	Dilute to 200 cc.
8.0	50 cc. M/5 KH_2PO_4	46.80 cc. M/5 NaOH	Dilute to 200 cc.

Boric acid. KCl-NaOH mixtures

7.8	50 cc. M/5 H_3BO_3 , M/5 KCl	2.61 cc. M/5 NaOH	Dilute to 200 cc.
8.0	50 cc. M/5 H_3BO_3 , M/5 KCl	3.97 cc. M/5 NaOH	Dilute to 200 cc.
8.2	50 cc. M/5 H_3BO_3 , M/5 KCl	5.90 cc. M/5 NaOH	Dilute to 200 cc.
8.4	50 cc. M/5 H_3BO_3 , M/5 KCl	8.50 cc. M/5 NaOH	Dilute to 200 cc.
8.6	50 cc. M/5 H_3BO_3 , M/5 KCl	12.00 cc. M/5 NaOH	Dilute to 200 cc.
8.8	50 cc. M/5 H_3BO_3 , M/5 KCl	16.30 cc. M/5 NaOH	Dilute to 200 cc.
9.0	50 cc. M/5 H_3BO_3 , M/5 KCl	21.30 cc. M/5 NaOH	Dilute to 200 cc.
9.2	50 cc. M/5 H_3BO_3 , M/5 KCl	26.70 cc. M/5 NaOH	Dilute to 200 cc.
9.4	50 cc. M/5 H_3BO_3 , M/5 KCl	32.00 cc. M/5 NaOH	Dilute to 200 cc.
9.6	50 cc. M/5 H_3BO_3 , M/5 KCl	36.85 cc. M/5 NaOH	Dilute to 200 cc.
9.8	50 cc. M/5 H_3BO_3 , M/5 KCl	40.80 cc. M/5 NaOH	Dilute to 200 cc.
10.0	50 cc. M/5 H_3BO_3 , M/5 KCl	43.90 cc. M/5 NaOH	Dilute to 200 cc.

Sørensen's standards are made as follows. The stock solutions are:

1. A carefully prepared exact tenth normal solution of HCl.
2. A carbonate-free exact tenth normal solution of NaOH.
3. A tenth molecular glycocoll solution containing sodium chlorid, 7.505 grams glycocoll and 5.85 grams NaCl in 1 litre of solution.
4. An M/15 solution of primary potassium phosphate which contains 9.078 grams KH_2PO_4 in 1 litre of solution.
5. An M/15 solution of secondary sodium phosphate which contains 11.876 grams $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ in 1 litre of solution.
6. A tenth molecular solution of secondary sodium citrate made from a solution containing 21.008 grams crystallized citric acid and 200 cc. carbonate-free N/1 NaOH diluted to 1 litre.

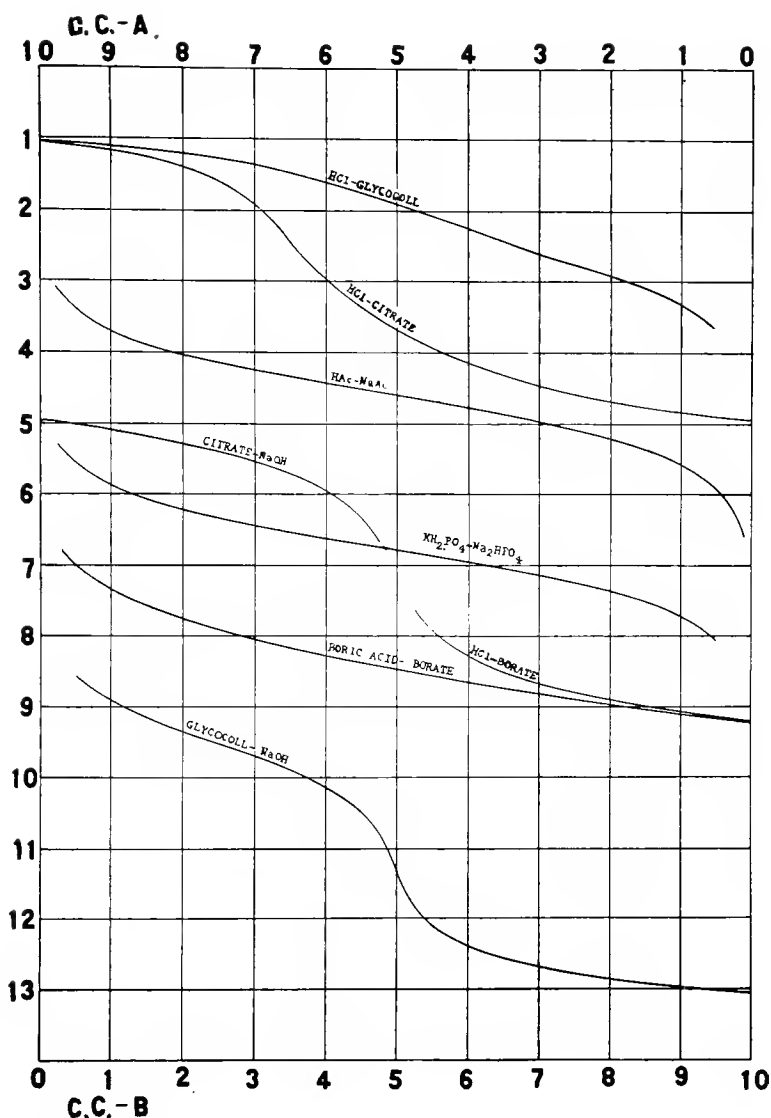


FIG. 11. SØRENSEN'S STANDARD MIXTURES, WALPOLE'S ACETATE SOLUTIONS
AND PALITZSCH'S BORATE SOLUTIONS

Mixtures of A parts of acid constituent and B parts of basic constituent.

7. An alkaline borate solution made from 12.404 grams boric acid dissolved in 100 cc. carbonate-free N/1 NaOH and diluted to 1 litre.

The materials for these solutions are described by Sørensen as follows.

The water shall be boiled, carbon dioxid free, distilled water, and the solutions shall be protected against contamination by CO_2 .

Glycocoll

Two grams glycocoll should give a clear solution in 20 cc. water and should test practically free of chlorid or sulfate. Five grams should yield less than 2 mgm. of ash. Five grams should yield, on distillation with 300 cc. of 5 per cent sodium hydroxid, less than 1 mgm. of nitrogen as ammonia. The nitrogen content as determined by the Kjeldahl method should be 18.67 ± 0.1 per cent.

Primary phosphate, KH_2PO_4

The salt must dissolve clear in water and yield no test for chlorid or for sulfate. When dried under 20 or 30 mm. pressure for a day at 100°C . the loss in weight should be less than 0.1 per cent, and on ignition the loss should be 13.23 ± 0.1 per cent. When compared colorimetrically with citrate mixtures the stock phosphate solution should lie between "7" and "8 citrate-HCl." On addition of a drop of tenth normal alkali or acid to 100 cc. the color of this phosphate solution with an indicator should be widely displaced.

Secondary phosphate, $\text{Na}_2\text{HPO}_4, 2\text{H}_2\text{O}$

The salt with this content of water of crystallization is prepared by exposing to the ordinary atmosphere the crystals containing twelve mols of water.⁶ About two weeks exposure is generally sufficient. The salt should dissolve clear in water and yield no test for chlorid or sulfate. A day of drying under 20 to 30 mm. pressure at 100°C . and then careful ignition to constant

⁶ Certain samples of secondary sodium phosphate sold for the preparation of buffer standards and called "Sørensen's Phosphate" are wrongly labeled Na_2HPO_4 .

weight, should result in a 25.28 ± 0.1 per cent loss. The stock solution should correspond on colorimetric test with "10 borate-HCl" and should be displaced beyond "8 borate-HCl" on addition of a drop of N/10 acid, and beyond "8 borate-NaOH" with a drop of alkali to 100 cc.

Citric acid, $C_6H_8O_7H_2O$

The acid should dissolve clear in water, should yield no test for chlorid or sulfate and should give practically no ash. The water of crystallization may be determined by drying under 20 to 30 mm. pressure at $70^\circ C$. On drying in this manner the acid should remain colorless and lose 8.58 ± 0.1 per cent. The acidity of the citric acid solution is determined by titration with 0.2 N barium hydroxid with phenolphthalein as indicator. Titration is carried to a distinct red color of the indicator.

Boric acid, H_3BO_3

Twenty grams of boric acid should go completely into solution in 100 cc. of water when warmed on a strongly boiling water bath. This solution is cooled in ice water and the filtrate from the crystallized boric acid is tested as follows. It should give no tests for chlorides or sulfates. It should be orange to methyl orange. A drop of N/10 HCl added to 5 cc. should make the filtrate red to methyl orange. Twenty cubic centimeters of the filtrate evaporated in platinum, treated with about 10 grams of hydrofluoric acid and 5 cc. of concentrated sulfuric acid and reevaporated, ignited and weighed, should yield less than 2 mgm. when corrected for non-volatile matter in the HF.

The following tables give the Sørensen mixtures with the corresponding pH values. Mixtures whose pH values are considered by Sørensen to be too uncertain and which he has indicated by brackets are omitted in these tables. The third decimal of Sørensen's tables are given by Sørensen in small type. The following tables are taken from the article in *Ergebnisse der Physiologie*, 12, 1912.

TABLE 7
Glycocoll mixtures (Sørensen)

GLYCOCOLL	HCl	pH
cc.	cc.	
0.0	10.0	1.038
1.0	9.0	1.146
2.0	8.0	1.251
3.0	7.0	1.419
4.0	6.0	1.645
5.0	5.0	1.932
6.0	4.0	2.279
7.0	3.0	2.607
8.0	2.0	2.922
9.0	1.0	3.341
9.5	0.5	3.679
	NaOH	
9.5	0.5	8.575
9.0	1.0	8.929
8.0	2.0	9.364
7.0	3.0	9.714
6.0	4.0	10.140
5.5	4.5	10.482
5.1	4.9	11.067
5.0	5.0	11.305
4.9	5.1	11.565
4.5	5.5	12.095
4.0	6.0	12.399
3.0	7.0	12.674
2.0	8.0	12.856
1.0	9.0	12.972
0.0	10.0	13.066

TABLE 8
Phosphate mixtures (Sørensen)

SECONDARY	PRIMARY	pH
<i>cc.</i>	<i>cc.</i>	
0.25	9.75	5.288
0.5	9.5	5.589
1.0	9.0	5.906
2.0	8.0	6.239
3.0	7.0	6.468
4.0	6.0	6.643
5.0	5.0	6.813
6.0	4.0	6.979
7.0	3.0	7.168
8.0	2.0	7.381
9.0	1.0	7.731
9.5	0.5	8.043

TABLE 9
Borate mixtures (Sørensen)

BORATE	HCl	pH
<i>cc.</i>	<i>cc.</i>	
5.25	4.75	7.621
5.5	4.5	7.939
5.75	4.25	8.137
6.0	4.0	8.289
6.5	3.5	8.506
7.0	3.0	8.678
7.5	2.5	8.799
8.0	2.0	8.908
8.5	1.5	9.007
9.0	1.0	9.087
9.5	0.5	9.168
10.0		9.241
	NaOH	
9.0	1.0	9.360
8.0	2.0	9.503
7.0	3.0	9.676
6.0	4.0	9.974
4.0	6.0	12.376

TABLE 10
Citrate mixtures (Sørensen)

CITRATE	HCl	pH
cc.	cc.	
0.0	10.0	1.038
1.0	9.0	1.173
2.0	8.0	1.418
3.0	7.0	1.925
3.33	6.67	2.274
4.0	6.0	2.972
4.5	5.5	3.364
4.75	5.25	3.529
5.0	5.0	3.692
5.5	4.5	3.948
6.0	4.0	4.158
7.0	3.0	4.447
8.0	2.0	4.652
9.0	1.0	4.830
9.5	0.5	4.887
10.0	0.0	4.958
	NaOH	
9.5	0.5	5.023
9.0	1.0	5.109
8.0	2.0	5.314
7.0	3.0	5.568
6.0	4.0	5.969
5.5	4.5	6.331
5.25	4.75	6.678
4.5	5.5	12.073
4.0	6.0	12.364

TABLE 11
Walpole's acetate buffer mixture, recalculated for intervals of 0.2 pH. Total acetate 0.2 normal

pH	CONCENTRATION (NORMALITY)	
	Acetic Acid	Sodium acetate
3.6	0.185	0.015
3.8	0.176	0.024
4.0	0.164	0.036
4.2	0.147	0.053
4.4	0.126	0.074
4.6	0.102	0.098
4.8	0.080	0.120
5.0	0.059	0.141
5.2	0.042	0.158
5.4	0.029	0.171
5.6	0.019	0.181

The stock solutions for the Palitzsch mixtures given in table 12 are an M/20 Borax solution containing 19.108 grams⁷ $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ in 1 litre; and an M/5 Boric acid, NaCl solution containing 12.404 grams⁷ H_3BO_3 and 2.925 grams NaCl in 1 litre.

TABLE 12
Palitzsch's borax-boric acid mixtures

M/20 BORAX	M/5 BORIC ACID	pH
cc.	cc.	
10.0	0.0	9.24
9.0	1.0	9.11
8.0	2.0	8.98
7.0	3.0	8.84
6.0	4.0	8.69
5.5	4.5	8.60
5.0	5.0	8.51
4.5	5.5	8.41
4.0	6.0	8.31
3.5	6.5	8.20
3.0	7.0	8.08
2.5	7.5	7.94
2.3	7.7	7.88
2.0	8.0	7.78
1.5	8.5	7.60
1.0	9.0	7.36
0.6	9.4	7.09
0.3	9.7	6.77

⁷ The values given by Palitzsch were calculated upon the basis of 11.0 as the atomic weight of boron. Since this was the value used, the new value of 10.9 given in the atomic weight table in the report of the international committee for 1920 should not be used in calculating the composition of the specific solutions given by Palitzsch.

CHAPTER VI

THE PROTEIN ERROR AND THE SALT ERROR IN COLORIMETRIC DETERMINATIONS

There are errors of technique such as incorrect apportionment of the indicator concentration in tested and standard solution and the use of unequal depths of solutions through which the colors are viewed that may be passed over with only a word of reminder. Likewise we may recall certain of the optical effects mentioned in Chapter II and then pass on to the more serious difficulties in the application of the indicator method.

In the correlation of electrometric and colorimetric measurements discrepancies have often been traced so clearly to two definite sources of error that they have been given categorical distinction. They are the so-called "protein" and "salt" errors.

From what has already been said in previous pages, it will be seen that, if there are present in a tested solution bodies which remove the indicator or its ions from the field of action either by absorption or otherwise, the equilibria which have formed the basis of our treatment will be disturbed. An indicator in such a solution may show a color intensity, or even a quality of color, which is different from that of the same concentration of the indicator in a solution of the same hydrogen ion concentration where no such disturbance occurs. We could easily be led to attribute very different hydrogen ion concentrations to the two solutions. This situation is not uncommon when we are dealing with protein solutions for in some instances there is distinctly evident the removal of the indicator from the field. In other cases the discrepancy between electrometric and colorimetric measurements is not so clear, nor can it always be attributed solely to the indicator measurement.

It is sometimes helpful to construct titration curves of a solution under examination, making measurements after addition of graded quantities of acid and alkali, in one case with the hydrogen electrode and in the other case with indicators, preferably indicators of different types. The indicator readings may then reveal

breaks not to be expected from the hydrogen ion relations of the solution. If, however, no comparison is made with hydrogen electrode measurements, the observer must rely to a considerable extent upon his judgment. "Protein errors" are generally the larger the more complex and concentrated the protein and tend to decrease with the products of hydrolysis.

If two solutions of inorganic material, each containing the same concentration of hydrogen ions, are tested with an indicator we should expect the same color to appear. If, however, these two solutions have different concentrations of salt, it may happen that the indicator color is not the same. As Sørensen (1909) and Sørensen and Palitzsch (1913) have demonstrated, this effect of the salt content of a solution cannot be tested by adding the salt to one of two solutions which have previously been brought to the same hydrogen ion concentration. The added salt, no matter if it be a perfectly neutral salt, will either change the hydrogen ion concentration or the hydrogen ion activity of the solution or so affect the electrode equilibrium that it appears as if the hydrogen ion activity is altered. So long as hydrogen electrode measurements are made the standard we must separate the "salt effect" into its influence upon the electrode potential and its effect upon the indicator. Tentatively we may regard the effect as being in each case of the same nature; on the one hand the salt altering the equilibria so that the hydrogen ion concentration is apparently increased and on the other hand the salt affecting the indicator equilibria themselves.

Bjerrum (1914) gives an example of a case where the influence of the neutral salt is evidently upon the buffer equilibrium rather than on the indicator. An ammonium-ammonium salt buffer mixture and a borate buffer mixture are both made up to the same color of phenolphthalein. On the addition of sodium chloride the color of phenolphthalein becomes stronger in the ammonium mixture and weaker in the borate mixture.

The following table taken from Prideaux (1917) illustrates the order of magnitude of the "salt error" in some instances.

INDICATOR	BUFFER USED	CHANGE OF pH IN PRESENCE OF 0.5 N NaCl
Para benzene sulphonic acid, azo naphthylamine.	Phosphate	-0.10
Para nitro phenol.....	Phosphate	+0.15
Alizarine, sulphonic acid.....	Phosphate	+0.26
Neutral red.....	Phosphate	-0.09
Rosolic acid.....	Phosphate	+0.06
Para benzene sulphonic acid, azo α -naphthol...	Phosphate	+0.12
Phenolphthalein.....	Phosphate	+0.12

In cases where the solutions under examination are of the same general nature hydrogen electrode measurements may be taken as the standard and colorimetric measurements calibrated accordingly. Sørensen and Palitzsch (1910) did this in their study of the salt errors of indicators in sea water. They acidified the sea water and passed hydrogen through to displace carbon dioxide, and then neutralized it to the ranges of various indicators with buffer mixtures and compared colorimetric with electrometric measurements. In this way they found the following corrections.

INDICATOR	BUFFER	PARTS PER 1000 OF SALTS AND CORRESPONDING ERRORS			
		35	20	5	1
Paranitro phenol.....	Phosphate	+0.12	+0.08		
Neutral red.....	Phosphate	-0.10	-0.05	0	0
α -naphthol phthalein...	Borate	+0.22	+0.17	+0.03	-0.07
	Phosphate	+0.16	+0.11	-0.04	-0.14
Phenolphthalein.....	Borate	+0.21	+0.16	+0.05	-0.03

If, for example, sea water of about 3.5 per cent salt is matched against a standard borate solution with phenolphthalein and appears to be pH 8.43 the real value is pH 8.22.

Such calibration is doubtless the very best way to deal with the salt errors since it tends to bring measurements to a common experimental system of reference.

In dealing with protein solutions calibration is less certain. When solutions to be tested vary greatly, not only in protein content but also in the composition and concentration of their salt content, systematic calibration becomes very difficult. When

there are added the difficulties presented by strong coloration and turbidity, calibration is impossible. Such is the situation to be faced when dealing with the media and the cultures which the bacteriologist must handle. We can bring to bear upon the problem no adequate explanation of the "salt effects," no general theory of the "protein errors," no comprehensive treatment of the optical difficulties, and finally no perfectly rigid basis upon which to compare the electrometric and colorimetric measurements. It seems wise to leave any detailed treatment of these subjects to painstaking research and to the resolution which will doubtless come when the conduct of strong electrolytes is placed upon a sound basis.

Such considerations should not deter us from choosing those indicators which give the most consistent values. When the agreement is good in a very wide variety of cases we may safely consider the method reliable for approximate determinations, without seeking to classify small discrepancies which may be observed.

The reader was warned in Chapter I that the treatment of the salt error of indicators would be lacking in specific treatment. There are various theories advanced, not only to account for the action of neutral salts in general but with particular reference to their action upon indicators. Sometimes they result in the establishment of more or less order among a series of cases; but then they appear either to fail or to involve assumptions the uncertainty of which liquidates the whole subject again. This is recognized by W. C. McC. Lewis when, in his comprehensive text (1916) he remarks: "An important field of investigation has not been discussed owing to the relatively small advance which has been made up to the present time as regards a sound theoretical basis, namely, the so-called neutral salt action."

There seems to be no way then to deal with either the protein or the salt error of indicators but to rely upon the use of those indicators which give *relatively* small errors, to keep in mind the order of magnitude of the error to be expected from the general nature of the solution tested, and, in important cases, to standardize to the electrometric basis as an arbitrary provisional standard.

Because of the great variety of solutions tested by the colorimetric method it is impracticable to give a condensed statement of the probable errors. Elaborate tables of colorimetric and

electrometric comparisons are given by Sørensen (1909) for the cases he studied. Clark and Lubs (1917) have tabulated their results with the sulphonphthalein indicators. Systematic studies of the salt errors remain to be made. Wells (1920) has studied cresol red in its relation to water tests, and Brightman, Meacham and Acree (1920) the effect of different concentrations of phosphate.

REFERENCES

- Abegg-Bose (1899), Arrhenius (1899), Bjerrum (1914), Chow (1920), Dawson-Powis (1913), Gillespie-Wise (1918), Harned (1915), Kolthoff (1916), Lewis (1912), McBain-Coleman (1914), McBain-Salmon (1920), Palmaer-Melander (1915), Poma (1914), Poma-Patroni (1914), Priedeaux (1917), Rosenstein (1912), Sackur (1901), Sørensen-Palitzsch (1910), (1913).

CHAPTER VII

APPROXIMATE DETERMINATIONS WITH INDICATORS

The distinctive advantage of indicators is the ease and rapidity with which they may be used to determine the approximate reaction of a solution. With the introduction of improved series of indicators, the charting of their ranges and better definition of distinctions in degrees of acidity and alkalinity, such terms as "slightly acid" or "neutral" are giving place to numerical values. Undoubtedly this will lead to niceties in analytical work and industrial processes that were previously overlooked. In many cases accuracy is unnecessary but good approximation is desirable. This may be attained by color memory without the aid of standard buffer solutions or even the system of bufferless standards to be described. To establish a color memory as well as to refresh it a set of "permanent" standards is convenient. These may be prepared with the standard buffer solutions in the ordinary way, protected against mold growth by means of a drop of toluol, and sealed by drawing off the test tubes in a flame or by corking with the cork protected by tinfoil or paraffine. For exhibition purposes long homeopathic vials make a very good and uniform container. They may be filled almost to the brim and a cork inserted, if a slit is made for the escape of excess air and liquid. The slit may then be sealed with paraffine. A hook of spring brass snapped about the neck makes a support by which the vial may be fastened to an exhibition board. A neater container is the so-called typhoid vaccine ampoule which is easily sealed in the flame.

If one of a series of standards so prepared should alter, the change can generally be detected by the solution falling out of the proper slope of color gradation. But if all in a series should change, it may be necessary to compare the old with new standards. Because such changes do occur, "permanent" standards are to be used with caution. The sulfon phthalein indicators make fairly permanent standards but the methyl red which is an important member of the series of indicators recommended by Clark and Lubs (1917) often deteriorates within a short time.

A device which furnishes a color standard to be interpreted by means of a dissociation curve is the color wedge of Bjerrum (1914). This is a long rectangular box with glass sides and a diagonal glass partition which divides the interior into two wedges. One compartment contains a solution of the indicator fully transformed into its alkaline form, the other a like concentration of the indicator transformed to the acid form. A view through these wedges should imitate the view of a like depth and concentration of the indicator transformed to that degree which is represented by the ratio of wedge thicknesses at the point under observation.

As an aid to memory the dissociation curves of the indicators are helpful even when used alone. The color chart shown in Chapter II is a still better aid to memory and within the limitations mentioned the colors may be used as rough standards.

Adjustment of bacteriological culture media. Perhaps no other science requires such continuous routine use of indicators as does bacteriology. This is chiefly in the adjustment of the "reaction" of culture media, but the use of indicators in bacteriology is by no means confined to this purpose alone.

In the old process of adjusting the "reaction" of culture media an aliquot of a given batch was titrated to the "first faint pink" with phenolphthalein. This was supposed to give the quantity of alkali required to bring the medium to "neutrality." Then, since experience had shown that a *particular* medium supported growth best when made more acid with a certain percentage acid reckoned from "neutrality," the required per cent of acid, less the difference between it and the equivalent of alkali required to reach "neutrality," was added to the main batch of medium. The result of this practice was that any change in the composition of the medium changed the final pH which a given per cent of added acid would induce. In some instances the difference was enormous. Now it is generally recognized that it is not only safer and more logical but easier to adjust on a pH basis. Just as the old procedure was carried out when adjustments were made to "the neutral point of phenolphthalein" so adjustments may be carried out on the new basis with only this difference—that an indicator is chosen which brings the "zero point" at the desired pH, as phenolphthalein brought it to the alkaline point of

about pH 8.4. Having thus attained the desired reaction it is left there without that addition of a certain "per cent of acid" which we now know sent the reaction into unknown regions.

If it is desired to adjust the medium to about pH 7.0, which is suitable for most saprophytes, adjustment to the *first faint* pink with phenol red will do. A reaction a little nearer "blood reaction" is attained by adjustment to the first faint ink with cresol red. Some pathogens are favored by adjustment to the first tinge of blue with thymol blue. A reaction which will suppress most bacteria and yet permit the growth of many molds is attained with brom phenol blue.

Testing of fermentations. Often the final pH of a medium is of greater significance than the quantity of acid or alkali formed. In the method of Clark and Lubs (1915, 1916) for the differentiation of the two main groups of the coli-aerogenes bacteria, as well as in the similar method of Avery and Cullen (1919) for separating streptococci, the composition of the medium is so adjusted to the metabolic powers of the organisms, that the reaction is left acid to methyl red in one case, and alkaline in the other. No exact pH measurements are necessary. In cases where large numbers of cultures falling within the range of one indicator are to be tested, the cultures may be treated with the indicator and compared by grouping. A careful determination made upon one member of a homogeneous group will serve for all. In this way large numbers of cultures may be tested in a day.

Indicator papers are to be avoided unless the use of an indicator solution is precluded. The subject is in a very unsatisfactory state and much remains to be done. Walpole's (1913) report upon experiments with litmus paper and Kolthoff's (1919) treatment have paved the way. If a paper is not sized, adsorption effects interfere, and if a paper is sized there is then the buffer effect of the sizing which obstructs the rapid attainment of equilibrium. There are occasions when the use of an indicator paper would be a distinct advantage but it must be used with caution or perhaps with calibration.

Colorimetric determination of hydrogen ion concentration without the use of standard buffer solutions

As mentioned on page 46 a knowledge of the conduct of indicators and especially of their apparent dissociation constants will permit the determination of hydrogen ion concentrations without the use of standard buffer solutions. If, for instance, an indicator conducts itself as a simple acid with dissociation constant 1×10^{-6} , we can construct the dissociation curve with its central point of inflection at pH 6, and then, assuming that this curve represents the relation of the percentage color transformation to pH, we can determine the pH of a solution if we can determine the percentage color transformation which this indicator displays in said solution. Proceeding on these simple and often unjustifiable assumptions we can now devise a very simple way of detecting the percentage color transformation. The following is quoted from Gillespie (1920):

We may assume that light is absorbed independently by the two forms of the indicator, and hence that the absorption, and in consequence of this the residual color emerging, will be the same whether the two forms are present together in the same solution or whether the forms are separated for convenience in two different vessels and the light passes through one vessel after the other. Therefore, if we know what these percentages are for a given indicator in a given buffer mixture, we can imitate the color shown in the buffer mixture by dividing the indicator in the proper proportion between two vessels, and putting part of it into the acid form with excess of acid, the rest into the alkaline form with excess of alkali.

Gillespie sets up in the comparator (see page 57) two tubes, one of which contains, for example, three drops of a given indicator fully transformed into the acid color, and the other of which contains seven drops of the indicator fully transformed into the alkaline form. The drop ratio 3:7 should correspond to the ratio of the concentrations of acid and alkaline forms of ten drops of the indicator in a solution of that pH which is shown by the dissociation curve of the indicator to induce a seventy per cent transformation. If then the two comparison tubes and the tested solution are kept at the same volume, and the view is through equal depths of each, a matching of colors should occur between the virage of the two comparison tubes and that of the tested solution.

Barnett and Chapman (1918) applied this method with the single indicator, phenol red. Gillespie (1920) extended the procedure to several other indicators and made use of the dissociation curves to smooth out to more probable values the relation of drop ratios to pH. Gillespie notes that the correspondence between the experimental results and the theoretical results predicted on the basis of the simplifying assumptions mentioned above is very good in the case of the sulfon phthalein indicators, chiefly because the two dissociation constants of these dibasic acids are so wide apart that the second dissociation constant with which the color transformation is related, is without serious interference from the first (compare also papers by Acree and his associates). In the case of phenolphthalein Gillespie showed that the application of the simple dissociation curve cannot be made because, as Acree (1908) has shown, the substance is a dibasic acid whose two dissociations seriously overlap.

It is important to note that Gillespie calls attention to discrepancies between the pH values corresponding to various drop ratios as determined by (1) Barnett and Chapman, (2) a report of the bacteriological committee (Conn-Harding-Kligler-Frost-Prucha and Atkins 1919) and (3) himself in the case of phenol red; and he puts forward the method (as did Barnett and Chapman) not as a precise one, but indicating its true values in these words:

The method should be of especial use in orienting experiments, or in occasional experiments involving hydrogen ion exponent measurements, either where it is unnecessary to push to the highest degree of precision obtainable, or where the investigator may be content to carry out his measurements to his limit of precision and to record his results in such a form that they may be more closely interpreted when a more precise study of indicators shall have been completed.

Gillespie cautions especially against comparisons at different temperatures without recording the temperatures. Were it not for the fact that the author has seen the method applied with total neglect of volume or concentration relations called for by the principle involved, it would seem unnecessary to add that the relations specified should be preserved in applying the method.

In table 13 are given the pH values corresponding to various drop ratios of seven indicators as determined by Gillespie. At the bottom of the table are shown the quantities of acid used to

obtain the acid color in each case. The use of acid phosphate instead of hydrochloric acid in two cases is because the stronger acid might transform the indicator into that red form which occurs with all the sulfon phthalein indicators at very high acidities. The 0.05 *M* HCl is prepared with sufficient accuracy by diluting 1 cc. concentrated hydrochloric acid (specific gravity 1.19) to 240 cc. The alkaline form of the indicator is obtained in each

TABLE 13
Gillespie's table of pH values corresponding to various drop-ratios

DROP-RATIO	BROM-PHENOL BLUE	METHYL RED	BROM-CRESOL PURPLE	BROM-THYMOL BLUE	PHENOL RED	CRESOL RED	THYMOL BLUE
1:9	3.1	4.05	5.3	6.15	6.75	7.15	7.85
1.5:8.5	3.3	4.25	5.5	6.35	6.95	7.35	8.05
2:8	3.5	4.4	5.7	6.5	7.1	7.5	8.2
3:7	3.7	4.6	5.9	6.7	7.3	7.7	8.4
4:6	3.9	4.8	6.1	6.9	7.5	7.9	8.6
5:5	4.1	5.0	6.3	7.1	7.7	8.1	8.8
6:4	4.3	5.2	6.5	7.3	7.9	8.3	9.0
7:3	4.5	5.4	6.7	7.5	8.1	8.5	9.2
8:2	4.7	5.6	6.9	7.7	8.3	8.7	9.4
8.5:1.5	4.8	5.75	7.0	7.85	8.45	8.85	9.55
9:1	5.0	5.95	7.2	8.05	8.65	9.05	9.75
Produce acid color with	1 cc. of 0.05M HCl	1 drop of 0.05M HCl	1 drop of 0.05M HCl	1 drop of 0.05M HCl	1 drop of 0.05M HCl	1 drop of 2 per cent H_2KPO_4	1 drop of 2 per cent H_2KPO_4

case with a drop of alkali (two drops in the case of thymol blue). Having described the comparator (see page 57) Gillespie proceeds as follows:

Test tubes 1.5 cm. external diameter and 15 cm. long are suitable for the comparator and for the strengths given for the indicator solutions. It is advisable to select from a stock of tubes a sufficient number of uniform tubes by running into each 10 cc. water and retainint those which are filled nearly to the same height. A variation of 3 to 4 mm. on a height of 8 cm. is permissible. Test tubes without flanges are preferable. The tubes may be held together in pairs by means of one rubber band per pair, which is wound about the tubes in the form of two figure 8's.

It is convenient to use metal test tube racks, one for each indicator, each rack holding two rows of tubes, accommodating one tube of each pair

in front and one in back. For any desired indicator a set of color standards is prepared by placing from 1 to 9 drops of the indicator solution in the 9 front tubes of the pairs and from 9 to 1 drops in the back row of tubes. A drop of alkali is then added to each of the tubes in the front row (2 drops in the case of thymol blue), sufficient to develop the full alkaline color and a quantity of acid is added to each of the tubes in the back row to develop the full acid color without causing a secondary change of color (see table 13 for quantities). . . . The volume is at once made up in all the tubes to a constant height (within about one drop) with distilled water, the height corresponding to 5 cc.

These pairs are used in the comparator and matched with the tested solution. This tested solution is added to ten drops of the proper indicator until a volume of 5 cc. is attained and the tube is then placed in the comparator backed by a water blank.

For further details see the original paper.

Dilution. As indicated in Chapter I a well buffered solution may often be moderately diluted without seriously altering the pH.

When dealing with complex solutions which are mixtures of very weakly dissociated acids and bases in the presence of their salts, and especially when the solution is already near neutrality dilution has a very small effect on pH, so that if we are using the crude colorimetric method of determining pH a five-fold dilution of the solution to be tested will not affect the result through the small change in the actual hydrogen ion concentration. Differences which may be observed are quite likely to be due to change in the protein or salt content. For this reason as well as for other reasons Clark and Lubs (1917) considered it wise to use M/20 standard comparison solutions instead of more concentrated standards for bacteriological media where dilution is often advantageous. The salt content of the standards undoubtedly influences the indicators and should be made as comparable as is convenient with the salt content of the solutions tested when these are diluted to obtain a better view of the indicator color.

The conclusion that dilution has little effect on the hydrogen ion concentrations of many solutions has long been recognized. Michaelis (1914) found little change in the pH of blood upon dilution, and Levy, Rowntree, and Marrott (1915) depended upon this *in part* in their dialysis method for the colorimetric determination of the hydrogen ion concentration of blood. Hen-

derson and Palmer (1913) have used the dilution method in determining the pH of urines, and Paul (1914) records some experiments with wines the pH values of which were affected but little by dilution. The legitimacy of dilution has been tacitly admitted by bacteriologists in their procedure of diluting media to be titrated to what is in reality a given pH as indicated by phenolphthalein.

In the examination of soil extracts colorimetrically little could be done were the "soil-solution" not diluted. Whatever may be the effect it is certain that the correlations between the pH values of such extracts and soil conditions is proving of great value (see Chapter XIX). Wherry has developed a field kit of the sulfon phthalein indicators with which he has found some remarkable correlations between plant distribution and the pH of the native soils. This field kit is now on the market.

Spotting. When only small quantities of solution are available or when highly colored solutions are to be roughly measured, their examination in drops against a brilliant white background of "opal" glass is often helpful. In the examination of colorless solutions, comparisons with standards may be made as follows. A drop of the solution under examination is mixed with a drop of the proper indicator solution upon a piece of opal glass. About this are placed drops of standard solutions and with each is mixed (by diffusion) a drop of the indicator solution used with the solution under examination. Direct comparison is then made (see Haas 1919).

Dr. L. D. Felton (private communication) has found this method invaluable in the examination of media for tissue cultures. He reports that a mixture of equal parts of methyl red and brom thymol blue furnished brilliant color contrasts in this drop method from pH 4.6 to pH 7.6.

CHAPTER VIII

OUTLINE OF THE ELECTROMETRIC METHOD

A noble metal coated with platinum black, which will hold large quantities of hydrogen, may be made to serve as a hydrogen electrode. When it is laden with hydrogen and immersed in a solution containing hydrogen ions, there is exhibited a difference of electrical potential between solution and electrode which is dependent upon the concentration of the hydrogen ions; just as the potential difference between a silver electrode and a solution of silver ions is dependent upon the concentration of the silver ions.

We have no reliable means of measuring this single potential difference; but when we join two hydrogen electrodes, as shown in figure 12, we can not only measure the difference between the aforementioned differences of potential, i.e., the total electromotive force (E. M. F.) of the "gas chain" as it is called, but we can also derive an equation showing how this E. M. F. will vary with the *ratio* of the concentrations of the hydrogen ions about the two electrodes. If C is the concentration of the hydrogen ions in one solution and C' the concentration in the other the E. M. F. of the combination will be related to the ratio of the concentrations by the following equation expressed in numerical form for a temperature of 25°C .

$$\text{E. M. F.} = 0.059 \log \frac{C}{C'}$$

If, then, we know one concentration and determine the ratio of the two from the E. M. F. by means of the above equation, we can calculate the other concentration.

There remains, however, a troublesome correction to make for the difference of potential which develops at L where the two unlike solutions are joined. This so-called liquid junction potential will be discussed in Chapter X. If it happens that the two electrodes are under unlike pressures of hydrogen there is also a cor-

rection to make for the inequality. This is the so-called barometric correction discussed in the next chapter.

Instead of the simple concentration chain illustrated in figure 12 it is more convenient to replace one of the electrodes with a calomel electrode, i.e. an electrode of mercury covered with HgCl in the presence of a definite concentration of KCl . This is a fairly stable and reproducible half-cell which can readily be connected with any solution whose hydrogen ion concentration we wish to

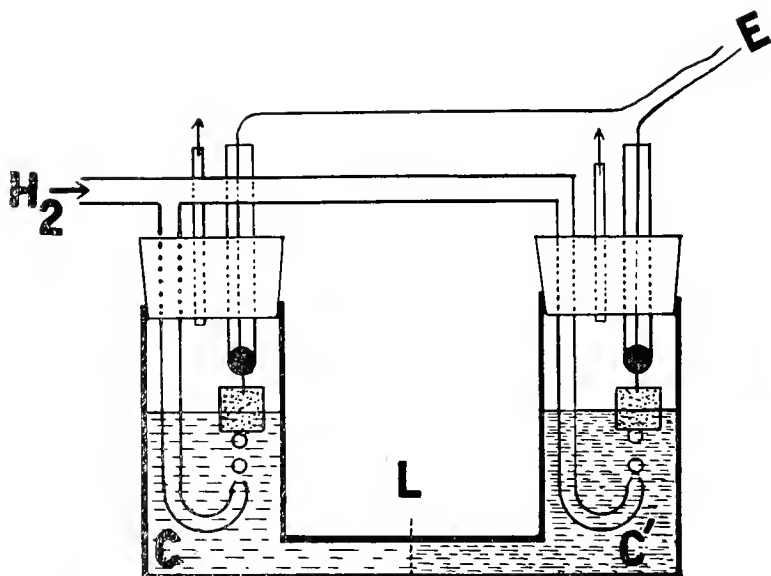


FIG. 12. DIAGRAM OF CONCENTRATION CHAIN OF HYDROGEN ELECTRODES

measure. But in this case, if we wish to apply the formula given above, we must so correct the total E. M. F. of the chain that the corrected E. M. F. will represent the potential difference between two hydrogen electrodes one of which is known. If the known, concentration C , is to be normal hydrogen ion concentration we must correct the total E. M. F. for the difference of potential between the calomel electrode and a hydrogen electrode immersed in a solution normal with respect to hydrogen ions.

If we assume that this has been determined, then the equation to apply becomes

$$\text{E. M. F.} - \left. \begin{array}{l} \text{difference of potential between calo-} \\ \text{mel and normal hydrogen electrode} \end{array} \right\} = 0.059 \log \frac{1}{C'}$$

Standard values for the difference of potential between a normal hydrogen electrode and various calomel half-cells will be found in the appendix and discussed in Chapter XVII.

CHAPTER IX

THEORY OF THE HYDROGEN ELECTRODE

In treating the theory of the hydrogen electrode we shall first consider Nernst's (1889) conception of electrolytic solution tension as a useful way of remembering certain important relations and then pass to the thermodynamic derivation of the E. M. F. of a concentration cell.

If a metal is placed in a solution of its salt there will be a difference of electrical potential between metal and solution which will vary in an orderly manner with the concentration of the metal ions. To account for the difference of potential Nernst assumed that a metal possesses a characteristic solution tension comparable with the vapor pressure of a liquid, or, better, with the solution pressure of a crystal of sugar—but with the important qualification that it is the metal ions which pass into solution. Imagine first that the metal is in contact with pure water. The metal ions passing into solution carry their positive charges and leave the metal negative. Thus there is established a so-called double layer of electrical charges at the interface between metal and solution, the solution being positively and the metal negatively charged relative to one another. This potential difference forcibly opposes further dissolution of metallic ions, for the relative positive electrical field in the solution and the relative negative field in the metal force back any further migration of positively charged bodies from the metal to the solution. Equilibrium is established when the electrostatic control equalizes the solution pressure.

If now there are already in the solution ions of the metal, the relative electrostatic field in the solution has already been partially established, fewer ions will escape from the metal and the metal is left more positive.

Therefore the higher the concentration of the metallic ions in the solution the more positive will be the charge on the metal and, conversely, the lower the concentration of the metallic ions in the solution the more negative will be the charge on the metal.

Not only metals but various gases are found to act in a similar way when means are devised to bring them into a situation as

easily handled as are metal electrodes. Hydrogen is one of these gases and the means of handling it as an electromotively active gas is to take it up in one of those metals such as platinum, palladium or iridium which in a finely divided condition hold large quantities of hydrogen. Platinum black deposited upon platinum and laden with hydrogen forms a hydrogen electrode. It can be brought into equilibrium with hydrogen ions as silver is brought into equilibrium with silver ions; and the more positive it becomes the higher must be the concentration of the positively charged hydrogen ions in the surrounding solution.

It remains however to formulate with mathematical precision the way in which the potential of the hydrogen electrode changes with the concentration of the hydrogen ions; and for this purpose the energy relations must be considered.

It is first *assumed*, as has been demonstrated for very dilute solutions, that the ions in solution obey the laws of gases. Let these laws therefore be applied in the following manner.

Suppose a metal electrode dips into a solution of ions of the same metal. Let the concentration of these ions be such that their partial pressure, which would be manifest in an arrangement for producing osmotic pressure, is P in the volume V .

Let the electrode be of such a size that one gram mol of ions, carrying nF faraday of electricity, can pass from electrode to solution to there raise the partial pressure by dP . The increase of the difference of potential between electrode and solution will be dE . The electrical work expended will then be $nFdE$ and the work taken up by the material system will be VdP . If the process is reversible, and the system is allowed to return to the original state,

$$nFdE - NdP = 0$$

From the gas laws $VP = RT$, or $V = \frac{RT}{P}$, whence

$$dE = \frac{RT}{nF} \frac{dP}{P}$$

By integration this becomes

$$E = \frac{RT}{nF} \ln^1 P + C \quad (18)$$

C is an integration constant.

¹ \ln is the symbol for the natural logarithm to the base e .

The integration constant is the point of reference for the general relation $E = \frac{RT}{nF} \ln P$. It is the potential difference between electrode and solution when some arbitrary unit of pressure is chosen and $P = 1$. Then in accordance with the unit chosen $E = C$. LeBlanc (1907) and others have substituted for C an equivalent constant of the form $-\frac{RT}{nF} \ln p$, called p the electrolytic solution tension of Nernst and so obtained the relation

$$E = \frac{RT}{nF} \ln \frac{P}{p}$$

But it is of doubtful value to postulate the physical composition of C in this manner. For present purposes we can afford to leave C as it stands, a pure integration constant.

Let us consider now the arrangement known as a concentration cell. Let the two vessels of figure 12 contain the same metal ion in concentrations C and C' corresponding to "osmotic pressures" P and P' . Let there dip into each solution an electrode of the metal. Let the two solutions be connected by a siphon, and the electrodes by a device for measuring the E. M. F.

Using the equation (18) developed above we know that at electrode 1 there will be a difference of potential $E = \frac{RT}{nF} \ln P + C$ and at electrode 2 a difference of potential $E' = \frac{RT}{nF} \ln P' + C$. The total E. M. F. will be the algebraic sum of these potential differences. If P' be less than P , the electrode in contact with the ions at partial pressure P' will be negative to the electrode in contact with the ions at partial pressure P . Hence

$$\text{E. M. F.} = E - E' = \frac{RT}{nF} \ln P + C - \left[\frac{RT}{nF} \ln P' + C \right] = \frac{RT}{nF} \ln \frac{P}{P'}$$

Since the ratio of the pressures may be considered equal to the ratio of the ion concentrations,

$$\text{E. M. F.} = \frac{RT}{nF} \ln \frac{C}{C'} \quad (19)$$

This is the fundamental equation for the E. M. F. of a concentration chain.

R is the gas constant, T the absolute temperature, $(273.09 + t \text{ centegrade})$, n the valency of the ion and F the faraday or the quantity of electricity associated with 1 gram molecule equivalent.

To put this equation into working form there have to be found the electrical equivalents for R and F. Since measurements of potential are to be made in terms of the international volt this and the related units will first be defined as they are given in Bureau of Standards Circular No. 60, (1916), "Electrical Units and Standards."

International ohm. The international ohm, which is generally referred to as the ohm, but which is to be distinguished as are other international units from the "absolute" units is defined as "the resistance offered to an unvarying electric current by a column of mercury at the temperature of melting ice, 14.4521 grams in mass, of a constant cross-sectional area and of a length of 106.300 cm."

International ampere. The international ampere, generally referred to as the ampere, is defined as "the unvarying electric current which, when passed through a solution of nitrate of silver in water in accordance with specification II (of the 1908 London Conference), deposits silver at the rate of 0.00111800 of a gram per second."

International volt. The volt is derived from current and resistance in accord with Ohm's law, $C = \frac{E}{R}$. The international

volt is therefore defined as "the electrical pressure (electromotive force) which, when steadily applied to a conductor the resistance of which is one international ohm, will produce a current of one international ampere."

F, the faraday, is derived for the international system as follows. The international ampere deposits silver at the rate of 0.00111800 of a gram per second. Since the atomic weight of silver is 107.88, a gram equivalent would be deposited in one second by 96494 amperes. The coulomb (international) is the quantity of electricity transferred by a current of one international ampere in one second. Hence 96494 coulombs are carried by a

gram equivalent of silver and this is the value of the faraday in the international system.²

Returning now to equation (19) we find that R , the gas constant, is derived from the gas equation

$$PV = \frac{P_0 V_0}{273.09} T, \text{ where } \frac{P_0 V_0}{273.09} \text{ is } R.$$

V_0 , the volume of 1 gram molecule of an ideal gas at one atmosphere pressure and 0°C . is 22412 ± 2 cc. (Berthelot, 1904). P_0 = one atmosphere or 76 cm. of mercury at 0°C . and 45° latitude. Since the acceleration of gravity at 45° latitude was taken to be 980.665 cm. per second when the "atmosphere" was defined, and, since 1 cc. mercury under the action of such a gravitational pull weighs 13.59545 grams, $P_0 = 980.665 \times 76 \times 13.59545$ or 1013276 dynes per square centimeter.

$$\text{Hence } R \text{ is } \frac{1013276 \times 22412}{273.09} = 83157719.8 \text{ ergs.}$$

10^7 ergs = one joule absolute. One joule, absolute = 0.99966 international joule. Hence $R = 8.3129446$ international joules, or volt coulombs.

From the derivations outlined above our equation reduces to the numerical form

$$E = \frac{8.3129446}{96494} \frac{T}{n} \ln \frac{C_1}{C_2}$$

Transposing to Briggsian logarithms (to the base 10) by dividing by 0.43429 we have

$$E = 0.00019837 \frac{T}{n} \log \frac{C_1}{C_2} \quad (20)$$

In the case of the hydrogen electrode, where the valence of the ionic hydrogen concerned is one, n is generally not written.

A table of the values of $0.00019837 T$ for various temperatures is given in the appendix.

² The absolute value is approximately 96,500 (Vinal and Bates, 1914).

The significance of the equation for the concentration chain is that, if T is known, the concentration of the ions in one solution can be determined from the E. M. F. of the chain if the concentration of the ions in the other solution is known. Fundamentally there is no other way of applying electromotive force determinations for the estimation of ion concentrations, unless there can be brought to bear mass action relations. This makes it necessary to start somewhere in the system with a solution whose hydrogen ion concentration has been determined by an independent method. Ordinarily however, a concentration chain of two hydrogen electrodes is not used, but rather a hydrogen electrode connected with a calomel electrode. But in this case there must be established the difference of potential between the calomel electrode and a known hydrogen electrode so that the E. M. F. of the new system may be corrected to give a potential difference as if between a known hydrogen electrode and the unknown. Then the formula for the concentration chain of two hydrogen electrodes may be applied.

If we express hydrogen ion concentrations in terms of normality, i.e., the grams of hydrogen ions in 1 litre of solution³ then the theoretical difference of potential between a hydrogen electrode in a solution normal with respect to the hydrogen ions and another hydrogen electrode in a solution of hydrogen ion normality C_x will be:

$$E = 0.00019837 T \log \frac{1}{C_x}$$

if C_x is less than normal as it usually is.

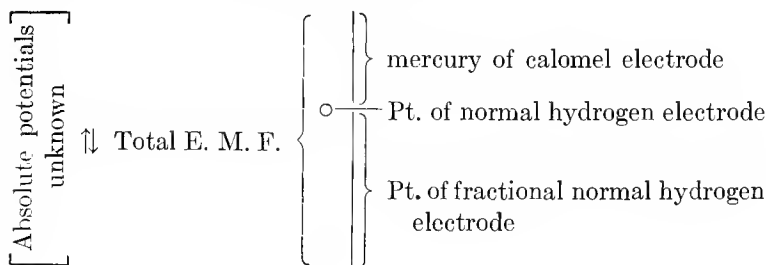
Unfortunately, however, we do not know how to prepare a solution normal with respect to the hydrogen ions. We are therefore forced to use some working standard such as the calomel electrode and to calculate the difference of potential between the calomel electrode and the *theoretical* normal hydrogen electrode from measurements made between the calomel electrode and a hydrogen electrode in some *fractional* normal hydrogen ion concentration. The resulting complexities make it very advantageous to preserve uniformity in some standard of reference potential and in the manner of using signs.

³ It makes little difference whether we regard the atomic weight of hydrogen as 1.0 or as 1.008 for the purpose at hand.

The standard of reference was formerly the potential difference between the mercury and the solution in the normal calomel electrode. This is still used by a few. The value 0.56 was given to this potential difference on the probability that it was the true value as established by Palmaer (1907). This value was questioned and, following the suggestion of Nernst (1897), another arbitrary standard has come into more general use. This is the potential difference between a hydrogen electrode under one atmosphere pressure of hydrogen and a solution normal with respect to the hydrogen ions. This potential difference is *defined* as zero. In the report of the Potential Commission of the Bunsen-Gesellschaft (Abegg, Auerbach and Luther, 1911) it is not specifically stated that this difference of potential shall be *zero at all temperatures*, but it seems to have been so understood and is specifically so stated by Lewis (1913). It is important to note that if the potential difference at the "normal hydrogen electrode" be taken as zero at all temperatures the temperature coefficients of electrodes referred to this standard may be very different from their *absolute* temperature coefficients.

If then the potential difference at the normal hydrogen electrode be taken as zero, the E. M. F. of a hydrogen electrode gas chain composed of a normal hydrogen electrode and a hydrogen electrode in hydrogen ion normality C_x will be the potential difference at this last mentioned electrode.

On lowering the hydrogen ion concentration the hydrogen electrode becomes more negative with respect to the solution. On the other hand the mercury of the calomel electrode is positive to the platinum of the normal hydrogen electrode. We therefore have the relation indicated diagrammatically below.



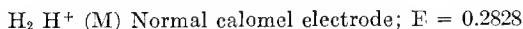
If we give a positive sign to the value of the potential difference between the mercury of the calomel electrode and the platinum of the normal hydrogen electrode this value must be subtracted from that of the total E. M. F. to give the potential difference between the platinum of the normal and the platinum of the fractional normal hydrogen electrode. This difference of potential is then used as shown on page 105 and we have the working formula.

$$\frac{\text{E.M.F. (observed)} - \text{E (calomel electrode)}}{0.0001983 \text{ T}} = \log \frac{1}{[\text{H}^+]} = \text{pH} \quad (22)$$

In actual experimental work with hydrogen electrode systems it is convenient to use the diagrammatic scheme shown above. However, the reader will encounter in the literature innumerable cases where the difference of potential between two electrodes is described simply as an "electrode potential," and where the sign given to the numerical value of what is really a difference of potential will differ according to the convention adopted.

Lewis, Brighton and Sebastian. for instance state: "the potential of the normal calomel electrode is -0.2828 " while LeBlanc says "the potential-difference between the calomel and the hydrogen electrode is equal to 0.283 volt." The difference in sign is due to the following difference in convention.

Lewis (1913) follows the rule that a positive sign given to a potential difference indicates the tendency of the positive current to run *through* a given cell from left to right when the cell is oriented as written. For instance,



indicates that the positive current runs through the cell from the normal hydrogen electrode to the mercury of the calomel electrode and back through the exterior wires to the normal hydrogen electrode. If the single potential difference between solution and hydrogen electrode is defined as zero then the single difference of potential between solution and mercury in the normal calomel electrode may be considered as -0.2828 since the mercury is negative to the solution with which it is in contact.

LeBlanc expresses the relation as follows;

$$E_{\text{Hg} \leftarrow \text{electrolyte}} = + 0.283$$

indicating that the positive current flows from the electrolyte to the electrode in the direction of the arrow.

In short the difference in sign amounts to ascribing to a *difference of potential* the relative sign of the electrolyte in the one case and the relative sign of the electrode in the other case.

The above equation is still incomplete because we have not taken into consideration the liquid junction potential differences which exist wherever two unlike solutions are brought into contact. Nor have we yet considered the effect upon the potential difference at a hydrogen electrode of a change in the pressure of hydrogen from the one atmosphere partial pressure specified for the normal hydrogen electrode. These two will be considered from the point of view of corrections to be made. Liquid junction potential differences, because of their distinct importance, will be treated in a separate chapter.

BAROMETRIC CORRECTION

The potential difference between a metal and solution will vary somewhat with the condition of the metal. A hammered, twisted or scratched electrode may show a different potential against a given concentration of its ions than will an electrolytically deposited metal. In the case of the hydrogen electrode it seems to make little difference whether the hydrogen be held in platinum, palladium or iridium but it does make a considerable difference if the surrounding pressure of hydrogen varies. If we have two hydrogen electrodes immersed in the same solution at the same temperature but under different pressures of gaseous hydrogen, we may assume that the concentration of the hydrogen in one electrode is different from that in the other electrode, and that the potential-difference may be expressed as

$$E = E_1 - E_2 = \frac{RT}{nF} \ln \frac{[H]_1}{[H]_2} \quad (23)$$

in which equation R , T , n , and F have their customary significance and $[H]_1$ and $[H]_2$ are concentrations of *atomic* hydrogen in the electrodes (platinum black). Since n , the valence of hydrogen, is 1, it may be omitted.

We may now assume that there is an equilibrium between the molecular hydrogen about the electrode and the atomic or ionic hydrogen in, or issuing from, the electrode. This equilibrium may be expressed in accordance with the mass law as follows:

$$\frac{[\text{H}] \times [\text{H}]}{[\text{H}_2]} = K_t \quad \text{where } [\text{H}] = \text{concentration of atomic hydrogen}$$

and $[\text{H}_2]$ = concentration of molecular hydrogen

Whence,

$$[\text{H}] = \sqrt{K_t[\text{H}_2]} \quad (24)$$

Substituting (24) in (23), we have

$$E = \frac{RT}{F} \ln \frac{\sqrt{K_t[\text{H}_2]_1}}{\sqrt{K_t[\text{H}_2]_2}} = \frac{RT}{2F} \ln \frac{[\text{H}_2]_1}{[\text{H}_2]_2}$$

It should be noted that the factor 2 in this equation does not come from giving hydrogen an effective valence of 2, as has often been stated, but from the introduction of equation (24). We might however derive the equation more directly by the energy relations and then the factor 2 would enter by reason of the volume change involved.

If the ratio of pressures is equal to the ratio of gas concentrations

$$E = \frac{RT}{2F} \ln \frac{P'_{\text{H}_2}}{P_{\text{H}_2}}$$

If P'_{H_2} be one atmosphere and P_{H_2} be expressed in atmospheres

$$E = \frac{RT}{2F} \ln \frac{1}{P_{\text{H}_2}} \quad (25)$$

This is the equation for the difference of potential between a hydrogen electrode under one atmosphere pressure of hydrogen (e.g. the normal hydrogen electrode) and a hydrogen electrode under pressure P_{H_2} .

Experimental justification of this equation is found in the experiments of Czepinski, Lewis and Rupert, Lewis and Randall, Lewis and Sargent, Ellis, Loomis and Acree and others.

Several writers have felt constrained to emphasize the fact that in determining the hydrogen pressure from barometer readings they have subtracted the vapor pressure of the solution. The emphasis is still advisable, for a considerable number of precise hydrogen electrode data are published with corrections for barometric pressure on the basis that the normal hydrogen electrode pressure is one atmosphere including the vapor pressure of the

solution. Corrections should be made to one atmosphere pressure of *hydrogen*, or else the standard used should be distinctly specified.

Clark and Lubs (1916) have suggested that a more consistent standard than that now recognized for the normal hydrogen electrode would be obtained by defining a standard *concentration* of hydrogen rather than a standard pressure. They used the commonly accepted "standard condition" of a gas which is the concentration at 0°C. and 760 mm. pressure. This would bring both the hydrogen and the hydrogen ions to a concentration basis whereas now the one is expressed in terms of concentration and the other in terms of pressure.

In applying the correction,

$$E_{\text{bar.}} = \frac{RT}{F} \ln \frac{1}{[P_{\text{H}_2}]},$$

it will be remembered that a decrease of the hydrogen pressure may be considered as a decrease of the electrolytic solution tension of the hydrogen. Hence under decreased hydrogen pressure the electrode is left more positive.

In the cell



if the hydrogen is under diminished pressure the E. M. F. of the cell is too low. Hence the correction must be applied to make the E. M. F. larger than observed.

$$\frac{\text{E. M. F.} + E_{\text{bar.}} - E_{\text{cal.}}}{0.00019837 T} = \text{pH} \quad (26)$$

To aid in the calculation of pressure corrections it is convenient to plot a curve giving the millivolts to be added to the observed E. M. F. for various corrected partial pressures. Tables of corrections from which a chart may be plotted are given in the appendix. In these tables the barometer pressures given are the corrected pressures. If hydrogen escapes from about the hydrogen electrode through a trap or other device which exerts back pressure, this pressure must be taken into consideration. Otherwise it is assumed that the pressure of the hydrogen is that of the barometer less the vapor pressure of the solution. To obtain the

corrected barometer reading the instrumental calibration of the instrument is first applied, then the temperature correction (a table of which is given in the appendix) necessary to bring the height of the mercury column at temperature t to its heights at temperature 0°C . Then there remains the correction for latitude (see tables in Landolt-Börnstein) in order that the pressure may be reduced to the common basis of the "atmosphere" namely, the pressure of 760 mm. mercury where the acceleration of gravity is 980.665 cm. per second.

For all ordinary cases it may be assumed that the vapor pressure is that of pure water at the temperature indicated.

If the unit pressure is one atmosphere the partial pressure must be reduced to atmospheres.

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CHAPTER X

POTENTIAL DIFFERENCES AT LIQUID JUNCTIONS

When two unlike solutions of electrolytes are brought into contact there develops at the junction a potential difference. Since no important chain can be constructed without involving such a liquid junction potential it is of great importance to know the cause so that the magnitude of the potential may be calculated or ways devised for its reduction.

The principal cause of the potential difference was attributed by Nernst to unequal rates of diffusion of ions across the plane of junction.

It has been found in the study of electrolytic conduction that under uniform potential gradient different ions move through a solution with different velocities. There are certain numbers representing the relative mobilities of the ions which are defined by the following relations. Let one faraday be passed between two electrodes. If the fraction N of one equivalent of anions has been transported from the cathode to the anode section of the solution $1-N$ fraction of one equivalent of the cation must have been transferred from the anode to the cathode section. The ratio of these two fractions is equal to the ratio of the absolute velocities of the ions.

$$\frac{N}{1-N} = \frac{\text{velocity of anion } (V_a)}{\text{velocity of cation } (V_c)}$$

Whence

$$N = \frac{V_a}{V_a + V_c} \text{ relative migration velocity of anion}$$

and

$$1-N = \frac{V_c}{V_a + V_c} \text{ relative migration velocity of cation.}$$

The following table taken from Lewis' *A system of physical Chemistry* gives the absolute and relative migration velocities of several ions.

ION	ABSOLUTE VELOCITY IN CENTIMETERS PER SECOND. 18°C.	MOBILITY
H.....	32.50 10^{-4}	318.00
K.....	6.70 10^{-4}	64.67
Na.....	4.51 10^{-4}	43.55
Li.....	3.47 10^{-4}	33.44
Ag.....	5.70 10^{-4}	54.02
OH.....	17.80 10^{-4}	174.00
Cl.....	6.78 10^{-4}	65.44
NO ₃	6.40 10^{-4}	61.78
CH ₃ COO.....	3.20 10^{-4}	35.00

Let it now be assumed that a solution of hydrochloric acid is placed in contact with pure water of negligible ion content at an imaginary plane surface. Independently of one another the chlorine and the hydrogen ions will *tend* to migrate across the interface and into the water. As shown in the above table the velocity of the hydrogen ion under the influence of a potential gradient is much greater than the velocity of the chlorine ion under the same gradient, and the relative velocities of free movement must therefore be in the same proportion. Consequently there will be established on the water side of the plane an excess positive charge. This charge will increase until the electrostatic attraction dragging the slower moving chlorine ions brings them to the velocity of the hydrogen ions. When this state is reached, as it is almost instantaneously, there is established a steady potential difference at the liquid junction. If the water is replaced by a solution of an electrolyte we have not only the chlorine and the hydrogen ions migrating across the boundary into this new solution but the ions of this solution migrating into the hydrochloric acid solution.

In the comparatively simple case where two solutions of different concentration of the same binary electrolyte are placed in contact the following elementary treatment may be used. Let the concentration of the ions on one side of the interface be C and on the other side be a lesser concentration C' .

When migration has established the steady potential E let it be over an interface of such extent that E is due to the separation of one faraday. If that fraction of the separated charge which is carried by the anion is n_a the work involved in the transport of n_a

equivalents from C to C' is $n_a RT \ln \frac{C}{C'}$. Likewise if that fraction of the charge carried by the cations is n_c the work involved in the transport of n_c equivalents from C to C' is $n_c RT \ln \frac{C}{C'}$. The work involved in the *separation* of the ions as they migrate from the high to the low concentration is

$$n_a RT \ln \frac{C}{C'} - n_c RT \ln \frac{C}{C'} = EF$$

Whence

$$E = (n_a - n_c) \frac{RT}{F} \ln \frac{C}{C'} \text{ or } (n_c - n_a) \frac{RT}{F} \ln \frac{C}{C'}$$

according to which ion moves the faster. Substituting for n_c and n_a the relative migration velocities

$$E = \frac{(V_a - V_c)}{(V_a + V_c)} \frac{RT}{F} \ln \frac{C}{C'} \quad (27)$$

Lewis and Sargent (1909) have treated the special case of two equally concentrated solutions of two binary salts having one ion in common. Substituting equivalent conductivities as proportional to mobilities they obtain

$$E = \frac{RT}{F} \ln \frac{\lambda_1}{\lambda_2} \quad (28)$$

where $\lambda_1 =$ and λ_2 are the equivalent conductivities of two solutions. Applying this equation they obtain the following correspondence between calculated and observed values of E, the liquid junction potential.

SOLUTIONS IN CONTACT	E (OBSERVED)	E (CALCULATED)	E (OBSERVED)-E (CALCULATED)
0.2N KCl-0.2N $KC_2H_3O_2$	-0.0080	-0.0082	0.0002
0.1N KCl-0.1N $KC_2H_3O_2$	-0.0074	-0.0077	0.0003
0.2N KCl-0.2N KOH.....	+0.0170	+0.0168	0.0002
0.1N KCl-0.1N KOH.....	+0.0165	+0.0165	0.0000
0.2N KCl-0.2N KBr.....	+0.0004	+0.0004	0.0000
0.2N NaCl-0.2N NaOH.....	+0.0192 \pm 0.0003	+0.0187	
0.1N KCl-0.1N HCl.....	-0.0286	-0.0286	0.0000

In the more general case limited chiefly by the condition that all the ions shall have the same valency Planck (1890) deduced the equation:

$$E = \frac{RT}{wF} \log_n \xi \quad (29)$$

where E is the contact difference of potential in volts and ξ is defined by the equation:

$$\frac{\xi U_2 - U_1}{V_2 - \xi V_1} = \frac{\log_n \frac{c_2}{c_1} - \log_n \xi}{\log_n \frac{c_2}{c_1} + \log_n \xi} \cdot \frac{\xi c_2 - c_1}{c_2 - \xi c_1} \quad (30)$$

c_1 is the sum of the concentrations of cations and anions in the more dilute solution and c_2 the sum in the more concentrated solution. w is the valency, R the gas constant, F the faraday, and

$$\begin{aligned} U_1 &= u'c' + u''c'' + \dots \\ V_1 &= v'c' + v''c'' + \dots \end{aligned}$$

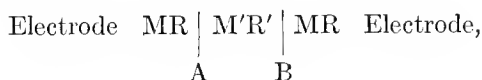
and U_2 and V_2 are similar sums for the second solution. The u' and v' symbols represent the ion mobilities and the c' symbols the corresponding ion concentrations.

Beside the limitation noted above this equation is strictly applicable only to very dilute solutions where dissociation is complete and it was deduced for the condition of a sharp boundary such as is not realized in experimental work.

P. Henderson (1907, 1908) therefore considered the connecting boundary as a series of mixtures of the two solutions in all proportions and deduced a somewhat simpler equation which Cumming (1912) has modified by introducing the mobilities at the different concentrations used.

It is of course obvious that the several equations which have been proposed are inapplicable when the solutions placed in contact are of unknown ion composition or very complex. They are therefore of no direct use in the study of concentration cells involving physiological fluids, although, as will be shown later, they are useful in defining certain relations which may be used in devising means for the reduction of the contact potential of physiological solutions. Even in simple cases, however, the applica-

bility of these equations is in some doubt because of the difficulty of maintaining experimentally the conditions for which they were set up. For instance Chanoz (1906) constructed the symmetrical arrangement:



and then, by maintaining a more or less sharp boundary at A by renewal of the contact, and allowing diffusion to occur at B, he obtained very definite E. M. Fs. instead of the zero E. M. F. which the symmetrical arrangement demanded. This time effect has been noted by Weyl (1905) and has since been frequently reported, for instance, by Bjerrum (1911) Lewis and Rupert (1911), Cumming and Gilchrist (1913) Walpole (1914) and Fales and Vosburgh (1918).

Since the change of potential has been ascribed to the diffusion and mixing which alter the distribution of the contending, migrating ions, it has seemed to many that the effect could be made more uniform and conditions more reproducible if the solutions were brought into contact at a membrane. This would tend to prevent mixing. Sand or other material would also delay the mixing and the diffusion. Cumming and Gilchrist (1913) used a symmetrical chain such as that of Chanoz (see above), and found that when a membrane was introduced at A while ordinary contact was allowed at B the symmetry of the chain was destroyed. Prideaux (1914) also found a difference when the contact was made in the one case with, and in the other case without, a parchment membrane. On considering this case and others in which the constituents of the membrane may take part in the establishment of the potential, he came to the conclusion that there were phenomena concerned which made the application of the ordinary equations of dubious value. See also Beutner (1913).

Lewis, Brighton and Sebastian (1917) using Bjerrum's (1911) suggestion of a layer of sand in which to establish the liquid contact found that "at no time were reproducible results obtained nor results which remained constant to 0.0001 volt for more than a minute or two. The potential of the liquid junction first established was surprising high (0.030 volt) and fell rapidly with-

out reaching any definite limiting value." The liquids placed in contact in this experiment were 0.1M HCl and 0.1M KCl. These authors abandoned the sand method.

On the other hand Myers and Acree (1913) report satisfaction with Bjerrum's "Sandfullung."

Other devices such as the use of a wick have been resorted to, but on the whole direct liquid contact is considered the best.

Recently Lamb and Larson (1920) have described the "flowing junction" which they find to be much more reproducible than the junctions usually made. They conclude "that a 'flowing' junction, obtained simply by having an upward current of the heavier electrolyte meet a downward current of the lighter electrolyte in a vertical tube at its point of union with a horizontal outflow tube, or by allowing the lighter electrolyte to flow constantly into a large volume of the heavier electrolyte, even with N solutions, gives potentials constant and reproducible to 0.01 of a millivolt." The device used by Lamb and Larson is illustrated in figure 13. It is encouraging to see experimental work such as that of Lamb and Larson being done upon this most difficult and most important phase of the subject.

A most important contribution to experimental methods of handling liquid junction potential differences arose from the theory of Nernst that the potential is due to the unequal migration of ions. The table of mobilities given on page 113 will show that if KCl is concerned no large potential can arise from the participation of its ions, because they have about the same mobility. If such a salt be present in high concentration upon both or even one side of the interface, the electrostatic fields of its ions will dominate the situation, and, migrating at equal velocities, will tend to maintain zero junction potential difference. Bjerrum (1911) studied the potential differences developed when concentrated solutions were thus employed and estimated the theoretical values with the aid of Planck's formula and with that of Henderson, which purports to take into account the effect of the destruction of a sharp boundary. He came to the conclusion that the use of a 3.5M KCl solution would not completely eliminate the potential against hydrochloric acid solutions but he suggested a more or less empirical extrapolation which would, he thought, give the proper correction. The correction is the difference in the

E. M. Fs. of a chain found when first 3.5M KCl is used and then when 1.75M KCl is used to connect two electrodes.

More recently Fales and Vosburgh (1918) have made an extensive comparison of various chains, and with the aid of Planck's formula to give the order of magnitude of various contact potentials, they have attempted to assign values which will lead to a general consistency. They concur with others in finding Planck's

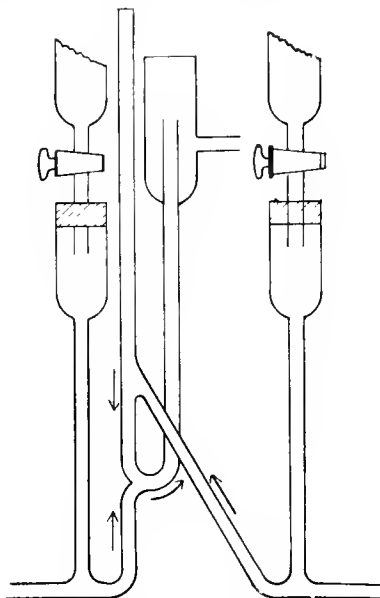


FIG. 13. LAMB AND LARSON'S DEVICE FOR THE FLOWING JUNCTION

formula invalid in the assignment of accurate values to liquid junctions, such as:

" x M KCl - 1.0M HCl and x M KCl - 0.1M HCl where x ranges from 0.1 to 4.1 and the temperature is 25°C."

They conclude that "there is no contact potential difference at 25° between a saturated solution of potassium chloride (4.1M) and hydrochloric acid solutions ranging in concentrations from 0.1 molar to 1.0 molar," confirming the suggestion of Loomis and Acree (1911).

Because of the great detail concerned in the reasoning of Fales and Vosburg it is impossible to briefly summarize their work, but

before their conclusion can be considered valid it must be noted that they themselves point out that "in an electromotive force combination having a contact potential difference as one of its component electromotive forces, the diffusion across the liquid junction of the one liquid into the other brings about a decrease in the magnitude of the contact potential difference, and this decrease may amount to as much as one-tenth of the initial magnitude of the contact potential difference." This experimental uncertainty undoubtedly renders questionable the *comparability*, if not the precision of measurements by different experimenters. If so there may lurk in the data used by Fales and Vosburg in their argument of adjustment to consistency an indefinite degree of incomparability.

Indeed the whole subject of contact potential is still in an unsatisfactory state. The experimental uncertainties which have been revealed have sometimes been overlooked in the calculation of important electrode values. Some of these values will be discussed in Chapter XVII. It now remains to determine if possible the order of magnitude of the contact differences of potential entering into chains used in the study of physiological solutions and the buffer solutions of the colorimetric method.

Since the concentrations of the hydrogen and the hydroxyl ions, which are the most mobile of all ions, are very low in most of these solutions, the contact potential difference may be expected to be much less than that found in hydrochloric acid solutions and similar solutions of high hydrogen or hydroxyl ion concentrations. It is the customary practice to employ saturated KCl in making the junction or to make the junction first with 3.5M, then with 1.75M KCl and extrapolate according to Bjerrum. The extrapolation so indicated generally amounts to only a few tenths of a millivolt, and in certain cases such as "standard acetate" to only 0.1 millivolt. Although such an extrapolation may be too low or too high its magnitude indicates that the error is not large. Furthermore there is found experimentally a drift in contact potential difference with time which is very much less than that found, for instance, at the junction sat. KCl-0.1M HCl. There can be no doubt that this is indicative of a low potential difference.

As pointed out by Clark and Lubs (1916), it is the difficulty in dealing with the contact potential of hydrochloric acid solutions

that renders them unsuitable for routine standardization of hydrogen electrodes.

Practical conclusions reached by experimentation are;

1. For precise E. M. F. measurements combinations having small liquid junction differences of potential should be used as far as is practicable.

2. It should be recognized that the E. M. F. of a cell which derives part of its E. M. F. from a liquid junction potential difference varies with the time elapsing after the formation of the liquid junction. Consequently this time should become a part of the data to be recorded.

3. It is preferable that measurements of E. M. F. be made directly after the formation of or the renewal of the liquid junction.

4. Since the liquid junction potential difference may vary with the manner of its formation the effort should be made to effect this junction in a reproducible way.

5. Reproducible potential differences are given by the flowing junction in the cases so far tried.

6. Narrow or capillary tubes at the point of liquid junction should be avoided.

7. An apparatus which permits the renewal of a junction and its complete removal when cells are left set up together for some time is preferable to any device such as membranes to protect the diffusion of solutions into electrode spaces.

8. Membranes at the liquid junction are to be avoided.

9. Wherever permissible saturated KCl solution should form one side of a liquid junction.

10. When a concentrated KCl solution is used to make liquid junction it should be stated whether the Bjerrum extrapolation with the use of 3.5M and 1.75M KCl has been employed or whether *saturated* KCl was used without the Bjerrum extrapolation.

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CHAPTER XI

HYDROGEN AND CALOMEL ELECTRODES AND ELECTRODE VESSELS

The form of an electrode must to some extent be adapted to the vessel in which it is to be used. For the most part the base of a hydrogen electrode consists simply of a piece of platinum foil welded to a platinum wire which is sealed into a glass tube carrying a mercury contact. It is advantageous to make such an electrode rugged as follows. Weld to a piece of platinum foil of about 1 sq. cm. a short length of no. 30 platinum wire by tapping the two smartly with the flat end of a punch while they are laid upon a flat hard surface in the white heat of a blast lamp. Draw off a glass tube to a *thin* blunt point and break away the capillary point till the no. 30 wire will enter. Slip the wire in till the foil touches the glass and holding the tube with foil uppermost apply a fine flame while rotating the tube. A perfect seal is made with a little of the glass adhering to the edge of the foil and holding it stiff. The stages are illustrated in figure 14.

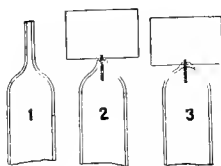


FIG. 14. CONSTRUCTION OF SIMPLE ELECTRODE

Electrodes made of platinum wire gauze are preferred by some investigators.

It is sometimes assumed that complete equilibrium can be attained only when the hydrogen in the interior of the metal supporting the platinum black is in equilibrium with that on the surface. To reduce the time factor of this soaking in process it is considered advantageous to use as the supporting metal a very thin film of platinum or iridium deposited upon glass. Doubtless the finest of such films could be deposited by holding the glass tangent to the Crookes' dark space of a vacuum discharge and

spattering the metal on from electrodes under 5000 volts difference of potential. The method practiced is to burn the metal on from a volatile solvent. The receipt given by Westhaver(1905) is as follows: 0.3 gram iridium chloride moistened with concentrated HCl is dissolved in 1 cc. absolute alcohol saturated with boric acid. To this is added a mixture of 1 cc. Venetian turpentine and 2 cc. lavender oil. The glass is dipped in this solution and rotated while drying to give an even deposit. It should then be very carefully dried to prevent blistering during the ignition. On gradually heating over an alcohol flame there is at last produced a very thin film of iridium. The process should be repeated until a good conducting film is obtained.

Gooch and Burdick (1912) have better success with a viscous mixture of pure chloroplatinic acid and glycerine. This is applied with an asbestos swab to the glass which has previously been heated to a temperature which will instantly volatilize the glycerine.

The chief technical difficulty in the preparation of electrodes with the films described is in establishing the necessary electrical connection. An exposed platinum wire contact destroys the object in using the film. Ordinarily the electrode is made by first coating a bar of glass in the end of which there is sealed a platinum wire and then fusing this into the end of a glass tube so that the platinum contact is exposed within the tube where mercury contact may be made. Connection with the film is made by the film of metal that runs through the glass seal. It is less clumsy to seal the wire into the end of a glass tube, break off the wire flush with the glass, coat the tube with the film and then close over the exposed wire with a drop of molten glass.

In the construction of such electrodes it is advisable to use a "hard" glass so that on heating the metallic film will not be fused into the glass and its conductivity lost. With a glass such as Pyrex the difference in its temperature coefficient and that of platinum must be taken into consideration. A very fine platinum wire may be sealed into Pyrex if the seal is of such a form as to have good mechanical strength.

A scheme which partially accomplishes the purpose of a thin film of supporting metal is to gold plate the platinum electrode, as gold is relatively impervious to hydrogen. There is another advantage in a gold plate to which reference will be made later.

According to the work of earlier investigations and the consensus of opinion among more recent investigators there seems to be no difference under equilibrium conditions between coatings of platinum, iridium or palladium black. No recent detailed data are available however. Of the three, iridium is recommended by Lewis, Brighton and Sebastian because of its higher catalytic activity, and palladium by Clark and Lubs (1916) for use in the study of physiological solutions because of the relative ease with which one deposit may be removed before the deposition of the next in the frequent renewals which are often necessary. Palladium black is easily removed by electrolysis in HCl. Deposits of platinum or iridium may be removed by electrolysis in HCl solution, if they are deposited upon a gold plate.

One of the essentials for making good deposits is a very high degree of cleanliness of the electrode. A good test is the evenness with which bubbles of hydrogen escape from the surface during electrolysis. Another essential in the preparation of a good electrode is that the deposit of black metal be not only even but of proper thickness. The inclination is to make the deposit too thick, with the production of a sluggish electrode. To obtain evenness of deposit it is necessary to hold down the dimensions of the electrode, provide more than one lead, or modify the rate of deposit. With this much said there remains very little systematized information upon the composition of solutions and the current densities which are best for the deposition of the finely divided metal required.

For the deposition of platinum black Ellis (1916) uses a solution of pure chloroplatinic acid containing 1 per cent Pt. He cautions against the use of the lead acetate which has come down to us in receipts for the deposition of platinum black upon electrodes for conductivity measurements. For the deposition Ellis uses a small auxiliary electrode and a current large enough to liberate gas freely at both electrodes. He continues the deposition with five-minute reversals of current for two hours and obtains a very thick coating. The author prefers an adherent, even, thin deposit sufficient to just cover the glint of metal beneath. In comparison of one against another in the same solution such thin deposits are found to agree within 0.02 millivolts. They may be deposited within a minute from the solutions used by the author.

For the deposition of iridium Lewis, Brighton and Sebastian (1917) make the gold or gold plated electrode the cathode in a 5 per cent solution of iridium chloride. "The best results were obtained with a very small current running for from twelve to twenty-four hours. Too large a current gives a deposit which appears more like platinum black and which is easily rubbed off."

The author has used deposits of platinum, iridium and palladium upon platinum and upon gold plated platinum. Acidified (HCl) 1 to 3 per cent solutions of the chlorides of each metal are used without much attention to the strength. The current from a four volt storage battery is allowed to produce a vigorous evolution of gas. The electrode is plunged, immediately after the deposition, into a dilute sulfuric acid solution and electrolyzed. It is required that the bubbles of hydrogen then escaping come off evenly, that the electrode be evenly covered with the deposit in thickness sufficient to cover the glint of polished metal, and that the deposit shall adhere under a vigorous stream of distilled water. If a solution does not deposit rapidly a little formic acid is added. No electrode is ever subjected to blast lamp treatment as is sometimes recommended. Instead, renewals are made by removing the old deposit by electrolysis in HCl solution, and, if any defect whatsoever develops to prevent a good redeposition after such electrolysis, the electrode is retired from duty.

There is needed a comprehensive study of conditions for electrode depositions.

For the gold plating of electrodes the following receipt may be used. Dissolve 0.7 gram gold chloride in 50 cc. water and precipitate the gold with ammonia water, taking care to avoid an excess. Filter, wash and dissolve immediately in a KCN solution consisting of 1.25 grams KCN in 100 cc. water. Boil till the solution is free from the odor of ammonia.

HYDROGEN ELECTRODE VESSELS

So many types of vessel have been published that it is difficult to do justice to the advantages of each. The selection must depend in some instances upon the material to be handled, but in any case there are a few principles which it is hoped will be made clear by a discussion of a few of the more widely used vessels.

The general method of operation is to partially or wholly immerse the electrode in the solution to be measured and then to bubble hydrogen through the vessel till constant potential is attained. The vessel described by Lewis, Brighton and Sebastian (1917) and illustrated in figure 15 is representative of the general type of vessel used for what may be called the classic mode of operation. The following is the quoted description of this vessel:

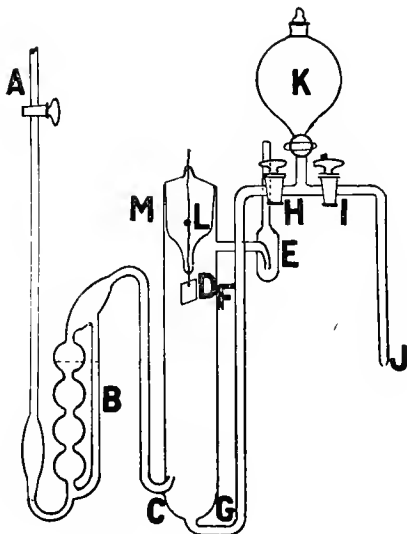


FIG. 15. HYDROGEN ELECTRODE VESSEL OF LEWIS, BRIGHTON AND SEBASTIAN

Hydrogen from the generator enters at A, and is washed in the bubbler B with the same solution that is contained in the electrode vessel. This efficient bubbling apparatus saturates the gas with water vapor, so that the current of hydrogen may run for a long period of time without changing the composition of the solution in the main vessel. The gas rises from the tip C, saturating and stirring the whole liquid from G to F, and leaves the apparatus through the small trap E, which also contains a small amount of the same solution. The platinum wire attached to the electrode D is sealed by lead glass into the ground glass stopper M. I is a joint made by fusing together the end of the platinum wire and the connecting wire of copper. The surface of the solution stands at the height F so that the iridium electrode is about one-half immersed. The apparatus from F through G, H, I to J is filled with the solution. With the form of construc-

tion shown it is an easy matter to fill the tube without leaving any bubbles of air. The reservoir K filled with the same solution serves to rinse out the tube I, J from time to time. The whole apparatus may be mounted upon a transite board, or for the sake of greater mobility, may be held in a clamp, the several parts being rigidly attached to one another to avoid accidental breakage. The whole is immersed in the thermostat about to the point L.

The tube J dips into an open tube through which communication is made to other electrode vessels. This connecting tube may be filled with the same solution as is contained in the hydrogen electrode vessel or with any other solution which is desired. All measurements with acids are made with one of the stopcocks H, I closed. These stopcocks are not greased and there is a film of acid in the closed stopcock which suffices to carry the current during measurement. In order to make sure that no liquid potential is accidentally established, the second stopcock may be closed up and the first opened. No difference of potential in acid solution has ever been observed during this procedure (but this is not true for solutions of salt and alkalies). If it is desired that both stopcocks be open, the same liquid that is in the electrode vessel is placed in the connecting tube at J and the stopcocks H and I are opened after the current of hydrogen has been cut off by the stopcock A, and the opening of the trap E has been closed.

If hydrogen enters the cell at the rate of one or two bubbles per minute several hours are required for the saturation of the solution and for the removal of air. After this time the potential is absolutely independent of the rate of flow of hydrogen and the generator may be entirely cut off for many hours without any change.

For some biochemical studies such a vessel is unsuitable. It is sometimes absolutely essential that equilibrium potentials be established rapidly. The necessity is perfectly apparent when one is dealing with an actively fermenting culture. It is not always so apparent when dealing with other solutions, but it is suspected that absolutely complete equilibrium is never attained in some complex biochemical solutions and that we have to depend upon speeding up the reaction between hydrogen and hydrogen ions till a virtual equilibrium point is attained (see Chapter XIV).

It was shown by Michaelis and Rona (1909) that a fairly constant E. M. F. is quickly attained, even in blood, if the platinized electrode, previously saturated with hydrogen, is allowed to merely touch the surface of the solution. This is probably due, as suggested by Hasselbalch (1913) and again by Konikoff (1913), to a rather sharply localized equilibrium at the point of contact. Reductions and gas interchanges having taken place within the small

volume at the point of contact, diffusion from the remaining body of the solution is hindered by the density of the surface layer with which alone the electrode comes in contact.

In exploring new fluids it appeared precarious to the writer to rely upon such a device, which appears to take advantage of only a localized and hence a pseudo-equilibrium, and which makes no allowance for a possible difference between the solution and surface film in the activity of the hydrogen ions. Hasselbalch's (1911) principle seemed therefore to be more suitable.

Hasselbalch found that a very rapid attainment of a constant potential can be obtained by shaking the electrode vessel. Under these conditions there should be not only a more rapid interchange of gas between the solution, the gaseous hydrogen, and the electrode, an interchange whose rapidity Dolezalek (1899) and Bose (1900) consider necessary, but the combined or molecular oxygen, or its equivalent, in the whole solution should be more rapidly brought into contact with the electrode and there reduced. Furthermore, by periodically exposing the electrode the hydrogen is required to penetrate only a thin film of liquid before it is absorbed by the platinum black. The electrode should therefore act more rapidly as a hydrogen carrier. For these reasons a true equilibrium embracing the whole solution should be rapidly obtained with the shaking electrode; and indeed a constant potential is soon reached.

Eggert (1914-1915) in Nernst's laboratory made a study of the rapidity of reduction by hydrogen electrodes in which he compared the effect of alternate immersion and exposure to the hydrogen atmosphere with the effect of continued immersion. In the reduction of metal salt solutions such as ferric salts he obtained a much greater velocity of reduction when the electrode was periodically removed from the liquid carrying a thin film of solution to be exposed to the hydrogen. The maximum velocity was proportional to the platinum surface and the time of contact with the gas. It was independent of the number of times per minute the electrode was raised and lowered. As the reaction neared completion the decrease in velocity of reaction became exponential.

Making use of the principles brought out in the preceding discussion and also certain suggestions noted in the chapter on liquid

junction potentials Clark (1915) designed a vessel which appears to have found favor for general use. A working drawing of this vessel is shown in figure 16. In figure 17 is a diagrammatic sketch of the complete system now in use by the author for ordinary work.

The electrode vessel is mounted in a clamp pivoted behind the rubber connection between J and H. This clamp runs in a groove

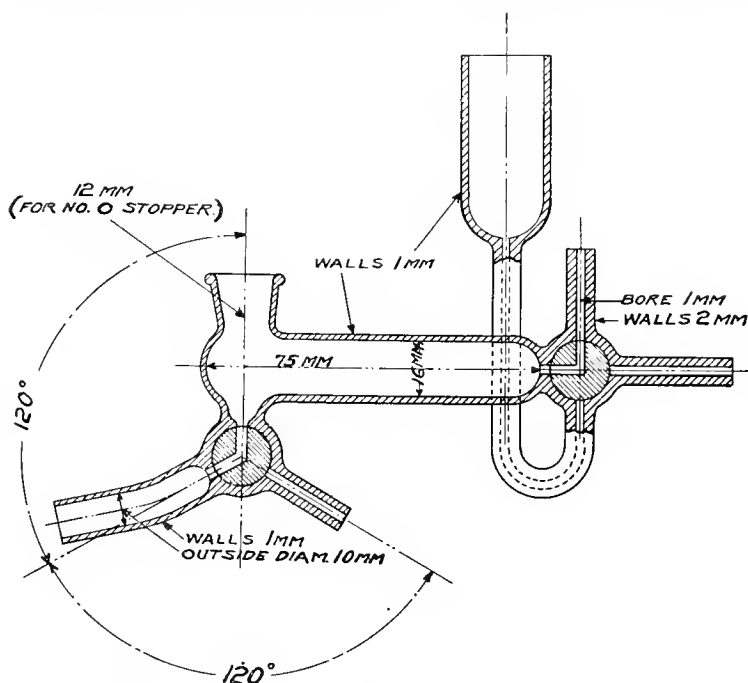


FIG. 16. A HYDROGEN ELECTRODE VESSEL (CLARK, 1915)

Notes. In submitting this working drawing to a glass blower it shall be specified that: (1) Cocks shall be joined to chamber with a neat and wide flare that shall not trap liquid. (2) Cocks shall be ground to hold high vacuum. (3) Bores of cock keys shall meet outlets with precision. (4) The handles of keys shall be marked with colored glass to show positions of bores. (5) Both handles of the keys shall be on the same side (front of drawing). (6) Vessel shall be carefully annealed. (7) Opening for no. 0 rubber stopper shall be smooth and shall have standard taper of the standard no. 0 stopper. (8) Dimensions as given shall be followed as closely as possible. (9) No chipped keys or violation of the above specifications shall be accepted.

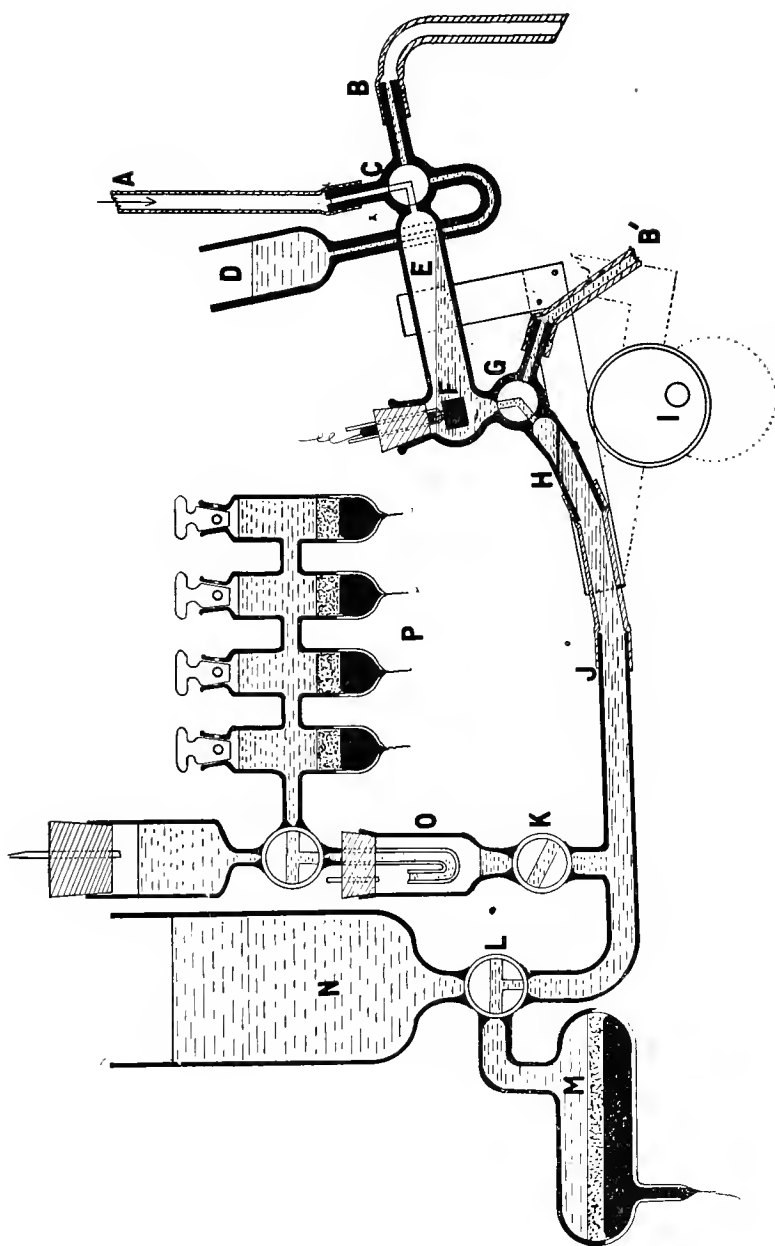


FIG. 17. A SYSTEM FOR THE MEASUREMENT OF HYDROGEN ELECTRODE POTENTIALS

of the eccentric I, the rotation of which rocks the vessel. In the manipulation of the vessel, the purpose is, first, to bring every portion of the solution into intimate contact with the electrode F and the hydrogen atmosphere to make use of the principle of alternate exposure and immersion of electrode and then, when equilibrium is attained, to draw the solution into contact with concentrated KCl solution and form a wide contact at H in a reproducible manner. The E. M. F. is measured directly after the formation of this liquid junction.

The vessel is first flooded with an abundance of hydrogen by filling the vessel as full as possible with water, displacing this with the hydrogen, and then flushing with successive charges of hydrogen from the backed-up generator. Water or solution is run into the vessel from the reservoir D which can be emptied through the drain B by the proper turning of the cock C. Solution or hydrogen displaced from the vessel is drained off at B'. These drains when they leave the electrical shielding (see p. 166) should hang free of any laboratory drain.

With the vessel rocked back to its lowest position the solution to be tested is run in from D (after a preliminary and thorough rinsing of the vessel with the solution) until the chamber E is about half full. Cock G is closed and cock C is turned so as to permit a constant pressure of hydrogen from A to bear upon the solution. For very careful work it is well to bubble hydrogen through the solution. The rocking is then commenced and continued until experience shows that equilibrium is attained with the solution of the type under examination. The eccentric I should give the vessel an excursion which will alternately completely immerse the electrode F and expose it all to the hydrogen atmosphere. The rate of rocking may be adjusted to obtain the maximum mixing effect without churning.

To establish the liquid junction the rubber tube between J and H is pinched while G is turned to allow KCl solution to escape at B'. Then a turn of G and the release of the pinch draws the solution down through the cock to form a broad mixed junction at H. For a new junction the old is flushed away with fresh KCl from the reservoir N by properly setting cock L.

With the closed form of calomel electrode, M, shown in the figure no closed stopcocks need be interposed between the terminals of

the chain. With the customary calomel electrode vessel it is necessary to use a closed cock somewhere and since this must be left ungreased it is well to have it a special cock¹ at J.

If a tube be led out from J and branched, several hydrogen electrode vessels may be joined into the system. At all events it is well to work with two vessels in parallel so that one may be flushed with hydrogen while the other is shaken.

The electrode F is supported in a sulfur-free rubber stopper. A glass stopper may be ground into place but is seldom of any advantage and may prove to be a mistake. In the first place it is advisable to be free with electrodes and to instantly reject any which fail to receive a proper coating of metal. The inclination to do this is less if it entails the rejection of a carefully ground stopper. Unless the stopper is accurately ground into place it is worthless. Furthermore it is very difficult to so grind a glass stopper that there will be left no capillary space to trap liquid. A rubber stopper can be forced into place without leaving such a space. The rapidity with which measurements are usually taken makes it improbable that a rubber stopper, if made sulfur-free, can have any appreciable effect. If the rubber must be protected a coating of paraffine will do.

The calomel electrode M is of the saturated type so that no particular care need be taken to protect it from the saturated KCl used in making junctions. This is the working standard for the accurate standardization of which there is held in reserve the battery of accurately made tenth normal calomel electrodes P. This battery may be connected with the system at any time by making liquid connection at O and opening K. After a measurement the liquid junction is eliminated and the space rinsed with the tenth normal KCl.

The design of this system is obviously for an air bath. The necessity of raising cocks out of an oil bath would not permit such direct connections as are here shown.

¹ To make an easily turning cock out of which KCl will not creep, grease the narrow part of the socket and the wide part of the key. When the key is replaced there will be two bands of lubricant on which the key will ride with an uncontaminated zone between for the film of KCl solution.

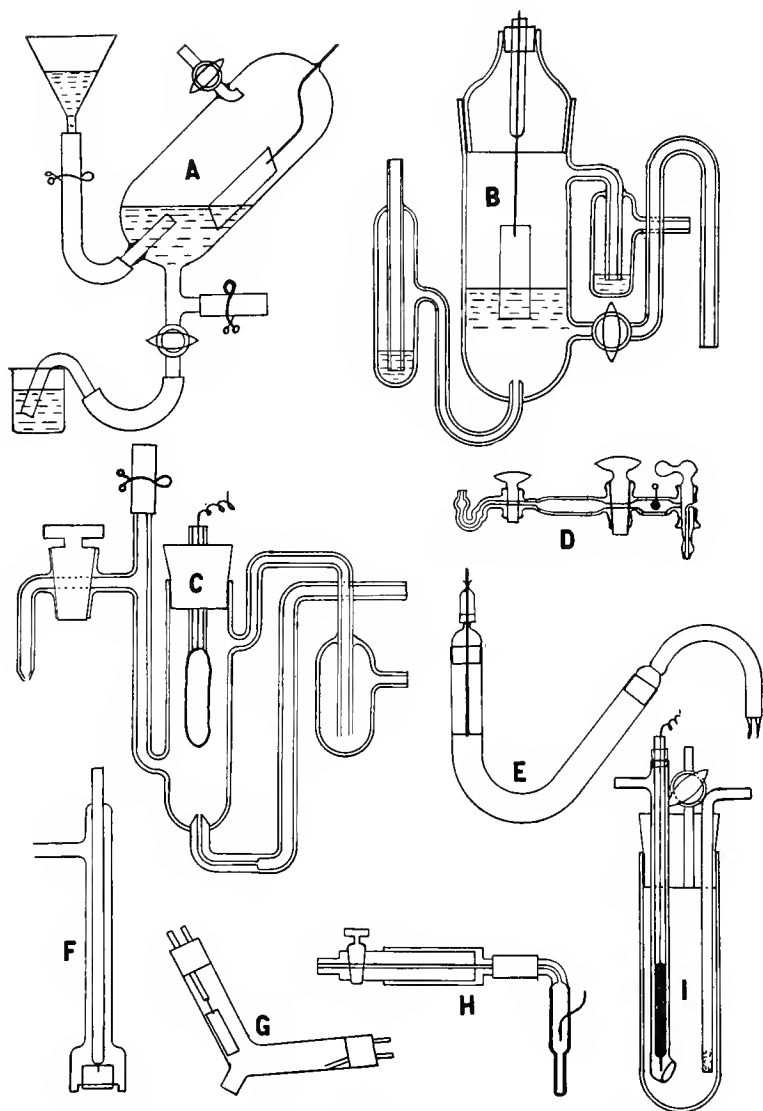


FIG. 18. TYPES OF HYDROGEN ELECTRODE VESSELS

In figure 18 are shown several other designs of electrode vessels. A is one of the original Hasselbalch vessels which have since been modified for the use of replaceable electrodes. B, (Sørensen), (Ellis) and C, (Walpole), are operated in a manner similar to the vessel shown in figure 15. Walpole's vessel was made of silica and the electrode of platinum film as described on page 121. D, (McClen-don and Magoon) was designed for determinations with small quantities of blood. E, (Michaelis), employs a stationary hydrogen atmosphere and a wick connection for the liquid junction. G, (Long) is a simple device which the designer thought applied the essential principles of Clark's vessel. Barendrecht's vessel, H, is designed for immersion in an open beaker for estimations during titrations. It is similar to a design of Walpole's (1914), but is provided with a plunger the working of which permits the rinsing of the bulb and the precise adjustment of the level of the liquid. Another immersion electrode is Hildebrand's, F, the successful operation of which depends upon a vigorous stream of hydrogen, which, on escaping from the bell surges the solution about the electrode. A modification which provides better protection of the electrode from oxygen is Bunker's design, I.

At this point it may be of interest to note that Wilke (1913) attempted to make a hydrogen electrode by using a thin tube of palladium on the interior of which hydrogen was maintained under pressure. One of the difficulties with such an electrode is the estimation of the hydrogen pressure at the solution-electrode interface. So far as the author knows Wilke's idea has never been developed to a practical point but it is worthy of study as an immersion electrode for industrial use.

CALOMEL ELECTRODES

Unless otherwise specified the calomel electrode is an electrode in which mercury and calomel is overlaid with a definite concentration of *potassium chloride*. For particular purposes HCl calomel electrodes or those containing some other chloride are used.

The general type of construction is shown by A, fig. 20. A layer of very pure mercury is covered with a layer of very pure calomel and over all is a solution of a definite concentration of KCl saturated with calomel.

The difference of potential between mercury and solution is determined primarily by the concentration of the mercurous ions supplied from the calomel. But, since there is equilibrium between the calomel, the mercurous ions and the chlorine ions, the concentration of the mercurous ions is determined by the chlorine ion content furnished by the KCl. One of three concentrations of KCl is usually employed—either 0.1 molecular, 1.0 molecular or saturated KCl. These are ordinarily referred to as the tenth normal, normal or saturated calomel electrodes.

The mercury used in the preparation of these electrodes or “half-cells” should be the purest obtainable. In Chapter XIII methods of purification are described. Sufficient mercury should be used to cover the platinum contact deeply enough to prevent solution reaching this contact on accidental shaking.

Some success has been attained with the use of the better grades of calomel supplied on the market but the risk is so great that it is best to prepare this material in the laboratory. A chemical and an electrolytic method will be described.

The chemical preparation of calomel. Carefully redistill the best obtainable grade of nitric acid. Dilute this slightly and with it dissolve some of the mercury prepared as described in Chapter XIII, always maintaining a large excess of mercury. Throw the solution into a large amount of distilled water making sure that the resulting solution is distinctly acid. Now, having distilled pure hydrochloric acid from a 20 per cent solution and taken the middle portion of the distillate, dilute and add it slowly to the mercurous nitrate solution with constant stirring. When the precipitate has collected, decant and treat with repeated quantities of pure distilled water (preferably conductivity water). The calomel is sometimes washed with suction upon a Buchner funnel but if due regard be taken for the inefficiency of washing by decantation it is preferable to wash repeatedly by decantation since there is thereby obtained a more even grained calomel. Throughout the process there should be present some free mercury.

Electrolytic preparation of calomel. Doubtless the better preparation of calomel is formed by electrolysis according to the method of Lipscomb and Hulett (1916). This is carried out in the same way that the mercurous sulfate for Weston cells is formed. For the preparation of mercurous sulfate Wolff and Waters (1907)

employ the apparatus shown in figure 19. An improvised apparatus may be made of a glass tube with paddles, platinum wire electrode and mercury contact and with two spools for bearing and pulley. In place of the sulfuric acid there is used normal hydrochloric acid. A direct current (from a four volt storage battery) must be used. The alternating current sometimes used

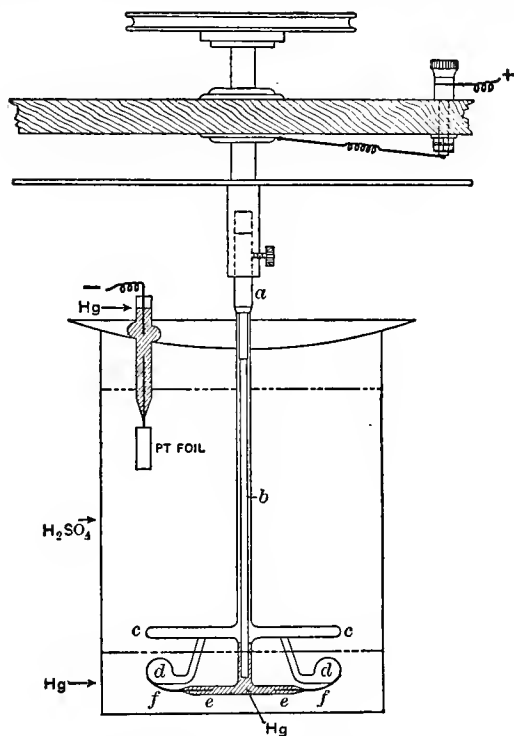


FIG. 19. WOLFF AND WATERS' APPARATUS FOR THE ELECTROLYTIC PREPARATION OF MERCUROUS SULFATE OR OF CALOMEL

in the preparation of mercurous sulfate does not seem to work in the preparation of calomel according to some preliminary experiments which Mr. McKelvy and Mr. Shoemaker of the Bureau of Standards kindly made for the writer. During the electrolysis the calomel formed at the mercury surface should be scraped off by the paddles *c* and *c* (fig. 19). The calomel formed by this process is heavily laden with finely divided mercury.

Calomel formed by either the chemical or the electrolytic process should be shaken with repeated charges of the KCl solution to be used in the half-cell before the calomel is placed in such a cell.

The variations in the potentials of calomel electrodes have been the subject of numerous investigations. Richards (1897) ascribed it partly to the formation of mercuric chloride. Compare Richards and Archibald (1902). Sauer (1904) on the other hand concluded that this had little to do with the inconstancy. Arguing upon the well known fact that the solubility of slightly soluble material is influenced by the size of the grains in the solid phase, Sauer thought to try the effect of varying the grain size of the calomel as well as the effect of the presence of finely divided mercury. With cells made up with various combinations he found the following comparisons:

Hg ⁻ (fine)	calomel (coarse)	against	calomel (fine)	Hg ⁺ (coarse)	= - 0.00287 volt
Hg ⁻ (fine)	calomel (coarse)	against	calomel (coarse)	Hg ⁺ (coarse)	= - 0.00037 volt
Hg ⁻ (coarse)	calomel (coarse)	against	calomel (fine)	Hg ⁺ (coarse)	= - 0.0025 volt

Lewis and Sargent (1909) state that they do not confirm Sauer in regard to the effect of the finely divided mercury but that they do confirm him in regard to the state of the calomel. These authors and others recommend that grinding the calomel with mercury to form a paste be avoided as this tends to make an uneven grain. It is better to shake the mercury and the calomel together but this is unnecessary if electrolytic calomel is used.

Here and there in the literature we find various other suggestions such as the elimination of oxygen from the cell but there seems to be no very substantial agreement in regard to this and several other matters as there is no substantial agreement in the preference of one concentration of KCl over another. By the use of carefully prepared material and the selection of the better agreeing members of a series, calomel electrodes may be reproduced to agree within 0.1 millivolt or better; but it has not yet been established whether or not this represents the order of agreement among electrodes made in different laboratories. The most extensive

comparison of calomel electrodes was made by Acree and his students (Myers and Acree, Loomis and Acree), but how far the reproducibility which they attained by short-circuiting the differences of potential is representative of the general reproducibility of such electrodes is not yet established.

In figure 20 are shown several calomel electrode vessels each with a feature that may be adapted to a particular requirement. Walpole's (1914) vessel, A, is provided with a contact that leads out of the thermostat liquid and with a three-way cock for flushing away contaminated KCl. A more elaborate provision for the protection of the KCl of the electrode is shown in the vessel of Lewis, Brighton and Sebastian (1917), B. A form useful as a saturated calomel electrode in titrations is shown at C. Fresh KCl passes through the U-tube to take the temperature of the bath and to become saturated with calomel shown at the bottom of this U-tube. D is Ellis' (1916) vessel, which in the particular form shown was designed to be sealed directly to the remainder of the apparatus used. A valuable feature is the manner of making electrical contact. Instead of the customary sealed-in platinum wire Ellis uses a mercury column. On closing the cocks the vessel may be shaken thoroughly to establish equilibrium. This feature has not been generally practiced and often it has been said that calomel electrodes should not be subjected to disturbance. Evidently the equilibrium is not established if disturbance can change the potential. Vessel E is a simple form useful for the occasional comparison electrode. It may be made by sealing the cock of an ordinary absorption tube to a test tube and adding the side arm. F is the vessel of Fales and Vosburgh (1918) with electric contact made as in the familiar Ostwald vessel (G).

In adding new KCl solution to a vessel it must be borne in mind that the KCl should be saturated with calomel before equilibrium can be expected. It is well therefore to have in reserve a quantity of carefully prepared solution saturated with calomel.

Lewis, Brighton and Sebastian (1917) state that certain grades of commercial KCl are pure enough to be used in the preparation of KCl solutions for the calomel electrode while other samples "contain an unknown impurity which has a surprisingly large effect upon the E. M. F. and which can only be eliminated by several recrystallizations." It is therefore obvious that the only

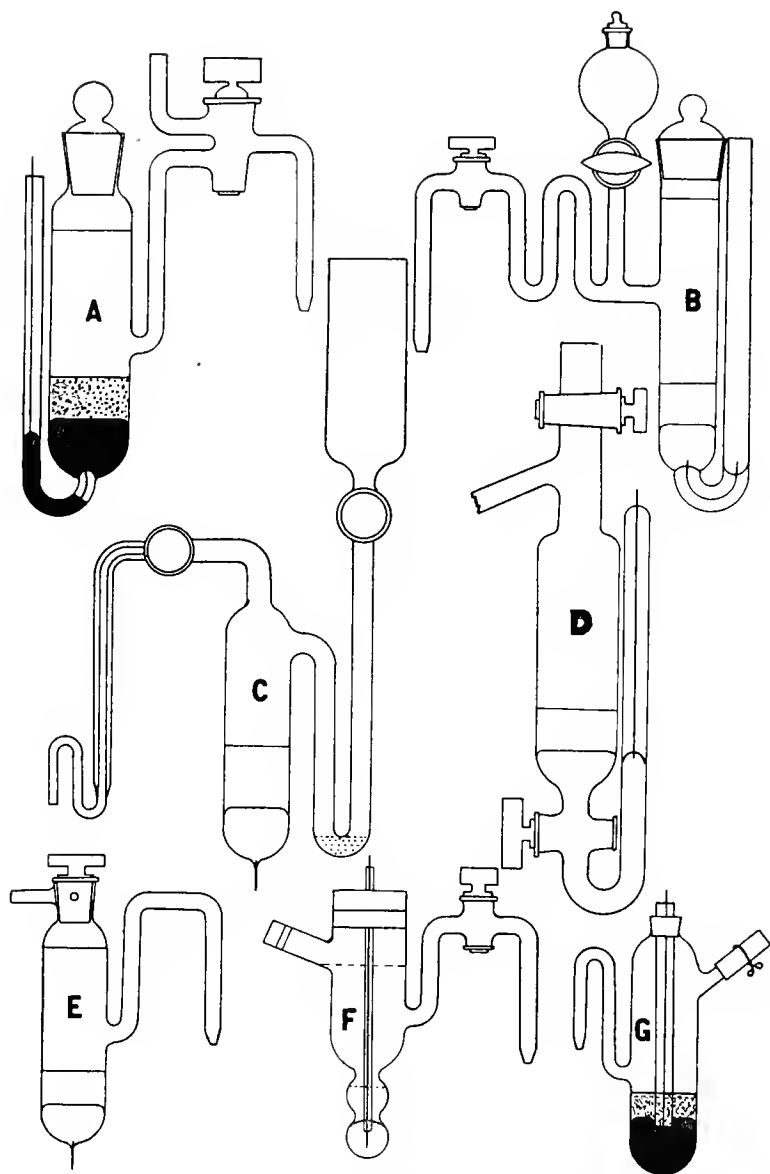


FIG. 20. TYPES OF CALOMEL ELECTRODE VESSELS

safe procedure, in lieu of careful testing by actual construction of electrodes from different material, is to put the best available KCl through several recrystallizations.

Acree has called attention to the possible concentration of the KCl solution by the evaporation of water and its condensation on the walls of vessels unequally heated in thermostats.

The values assigned to the potential differences at the several calomel electrodes at different temperatures vary. A judicious selection will wait upon the consideration of several important matters. Some of the more important of these will be presented in Chapter XVII. At this point however we may recount without comment some of the more frequently used values which the reader may choose to use.

Clark and Lubs (1916) give the following compilation of Bjerrum's values and those of Sørensen and Koefoed published by Sørensen (1912):

AUTHOR	TEMPERATURE	POTENTIAL DIFFERENCE BETWEEN NORMAL HYDROGEN ELECTRODE AND N/10 CALOMEL ELECTRODE WHEN HYDROGEN PRESSURE IS	
		One atmosphere less vapor pressure	One atmosphere
	°C.	volts	volts
Bjerrum.....	0	0.3366	0.3367
Sørensen and Koefoed.....	18	0.3377	0.3380
	20	0.3375	0.3378
Bjerrum.....	25	0.3367	0.3371
Sørensen and Koefoed.....	30	0.3364	0.3370
	40	0.3349	0.3359
	50	0.3326	0.3344
	60	0.3290	0.3321
	75	0.3243	0.3315

In the report of the "Potential Commission" of the Bunsen-Gesellschaft (Abegg, Auerbach and Luther, 1911) the normal hydrogen electrode standard of difference of potential was adopted. This of course is only incidental except as temperature coefficients enter. The differences of potential between the normal hydrogen

electrode and the tenth normal and normal KCl calomel electrodes were given as 0.337 and 0.284–0.283 respectively. Auerbach (1912) in a review of this report called attention to the smaller temperature coefficient of the potential difference at the tenth normal calomel electrode when referred to the normal hydrogen electrode (as having zero potential difference at all temperatures) and suggested that the tenth normal electrode be taken as the working standard with the value 0.3370 between 20°C and 30°C

Michaelis and Davidoff (1912) introduced the saturated KCl calomel electrode. Michaelis (1914) gives the following table of values for the potential differences referred to the normal hydrogen electrode for the tenth normal and the saturated calomel electrodes.

TEMPERATURE	TENTH NORMAL	SATURATED
15		0.2525
16		0.2517
17		0.2509
18	0.3377	0.2503
19		0.2495
20	0.3375	0.2488
21		0.2482
22		0.2475
23		0.2468
24		0.2463
25		0.2458
30	0.3364	
37		0.2355
38	0.3355	0.2350
40	0.3349	
50	0.3326	
60	0.3290	

Loomis and Acree (1911) present a choice of values for the tenth normal calomel electrode at 25°C. referred to the normal hydrogen electrode. The choice depends upon the ionization ascribed to the hydrochloric acid solutions used in their hydrogen electrodes and upon the values of the contact differences of potential which were involved. Loomis (1915) is inclined to accept the value 0.3360.

In 1914 Lewis and Randall applied "corrected degrees of dissociation" to the hydrochloric acid solutions used in arriving at the difference of potential at 25° between calomel electrodes and the theoretical normal hydrogen electrode. Defining the normal calomel electrode as the combination $\text{Hg} \mid \text{HgCl}_2, \text{KCl (1M)}, \text{KCl (0.1)}$ they reach the value 0.2776. The difference of potential between this electrode and the tenth normal they give as 0.0530. Whence the value for the tenth normal electrode is 0.3306. These values were revised by Lewis, Brighton and Sebastian (1917) to 0.2828 for the difference of potential between the normal calomel and the normal hydrogen electrode, and 0.0529 for the difference between the normal and the tenth normal.

Michaelis and Davidoff (1912) have called attention to the advantages of the saturated KCl calomel electrode as a working standard. It does not require the very careful protection from contamination by the saturated KCl generally used in making liquid junctions that is required in the use of normal and tenth normal electrodes. Furthermore the relatively high conductance of the saturated KCl in the connecting tube permits a fuller use of the sensitivity of low resistance galvanometers.

As described by Michaelis the saturated calomel differs in no essential way from other calomel electrodes except in the concentration of the KCl. It has not yet been subjected to as extensive accurate investigation as the tenth and the normal calomel electrodes and therefore had best be used for the present as a working standard only.

The values preferred by the author are given in Chapter XVII.

SUPPLEMENTARY REFERENCES

Abegg (1902), Auerbach (1912), Bjerrum (1911), Bugarszky (1897), Clarke-Myers-Acree (1916), Coggeshall (1895), Coudres (1892), Ellis (1916), Fales-Vosburgh (1918), Lewis-Brighton-Sebastian (1917), Lewis-Sargent (1909), Lipscomb-Hulett (1916), Loomis-Acree (1911), Loomis-Meacham (1916), Michaelis (1914), Michaelis-Davidoff (1912), Myers-Acree (1913), Newberry (1915), Richards (1897), Richards-Archibald (1902), Sauer (1904), Steinwehr (1905), Walpole (1914), See also Chapter XVII for potential values.

CHAPTER XII

THE POTENTIOMETER AND ACCESSORY EQUIPMENT

The method usually employed in the measurement of potential differences is the Poggendorf compensation method, the potentiometer method. In principle it consists in balancing the potential difference under measurement against an opposing, known potential difference. When the unknown is so balanced no current can flow from it through a current indicating instrument such as a galvanometer.

The principle may be illustrated by the arrangement shown in figure 21 which is suitable for very rough measurements.

According to modern theory the conduction of electricity in metals is the flow of electrons, the electron being the unit electrical charge. By an unfortunate chance the two kinds of electricity, which were recognized when a glass rod was rubbed with silk, were given signs (+ for the glass and - for the silk) which now leave us in the predicament of habitually speaking of the flow of positive electricity when the evidence is for the flow of negative charges, the electrons. But so far as the illustration of principles is concerned it makes little difference and we shall choose to deal with the negative charges in order to make free use of a helpful analogy. We may imagine the electrons, already free in the metal of our electrical conductors, to be comparable with the molecules of a gas which if left to themselves will distribute themselves uniformly throughout their container (the connected metallic parts of our circuits). We may now imagine the battery S (figure 21) as a pump maintaining a flow of gas (electrons) through pipes (wires) to R to A to B and back to S. The pipe (wire) AB offers a uniform resistance to the flow so that there is a uniform fall of pressure (potential) from A to B while the pump (battery) S maintains a uniform flow of gas (electrons). If we lead in at C and at D the ends of the pipes (wires) from another pump (battery) X, taking care that the high pressure pipe (wire) from X leads in on the high pressure side of AB, we can move C, D or both C and D until they span a length of AB such that the difference of pressure

(difference of potential) between C and D on AB is equal and opposite to the difference of pressure (difference of potential) exerted between C and D by X. Then no current can flow from X through the current indicating instrument G and we thereby know that balance is attained.

If we know the fall of electrical potential per unit length along AB the difference of potential exerted by X will be known from the length of wire between C and D. We now come to the manner in which this fall of potential per unit length is determined.

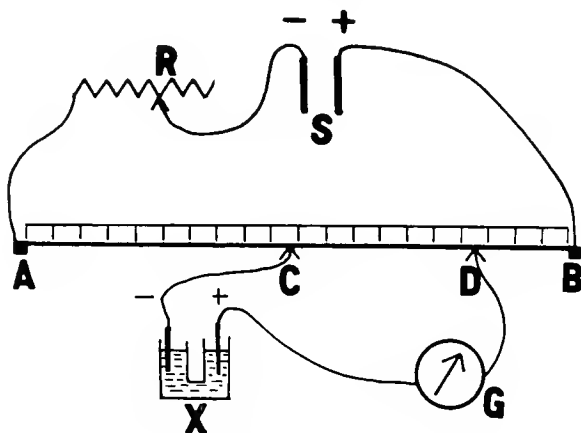


FIG. 21. ELEMENTARY POTENTIOMETER

Choosing for units of electrical difference of potential, electrical resistance and electrical current, the volt, the ohm, and the ampere respectively, we find that the relation between difference of potential, current and resistance is expressed by Ohm's law:

$$\text{Current (in amperes)} = \frac{\text{Difference in potential (in volts)}}{\text{Resistance (in ohms)}}$$

Or

$$C = \frac{E}{R}$$

With this relation we could establish the fall of potential along AB by measuring the resistance of AB and the current flowing. But this is unnecessary, for we have in the Weston cell a standard

of electromotive force (E. M. F.) which may be directly applied in the following manner. The unknown x (figure 21) is switched out of circuit and in its place is put a Weston cell of known E. M. F. Adjustment of C and D is made until the "null point" is attained, when the potential difference between the new positions of C and D is equal to the E. M. F. of the Weston cell. From such a setting the potential fall per unit length of AB is calculated. It must be especially noted however that for such a procedure to be valid the current in the potentiometer circuit must be kept *constant between the operations of standardization and measurement* for the fundamental relationship upon which reliance is placed is that of Ohm's law $C = \frac{E}{R}$. It will be noted that the establishment of the difference

of potential between any two points on AB by the action of S and the resistance of AB is strictly dependent upon the relation given by Ohm's law, $C = \frac{E}{R}$; but, since we draw no current

from x when balance is attained, the resistance of its circuit is of no fundamental importance. It only affects the current which can flow through the indicating instrument G when the potential differences are out of balance. It is therefore concerned only in the sensitivity of G.

The simple potentiometer system described above is susceptible to both refinement in precision and convenience of operation.

With the inevitable variations in the potentiometer current which occur as the battery runs down it would be necessary to recalculate from moment to moment the difference of potential per unit length of the wire AB if the procedure so far described were used. This trouble is at once eliminated if the contacts of the Weston cell can be thrown in at fixed points and the current is then adjusted by means of the rheostat R so that there is always *the same* uniform current producing, through the resistance between the Weston cell contacts, the potential difference of this standard cell. Having thus arranged for the adjustment of a uniform current at all times and having the resistance of AB already fixed it is now permissible to calibrate the wire AB in terms of volts.

In the Leeds and Northrup potentiometer (fig. 22), the resistance AB of our elementary instrument (fig. 21) is divided into two

sections one of which A-D (fig. 22) is made up of a series of resistance coils between which M makes contact and the other portion of which is a resistance wire along which M' can slide. When the potentiometer current has been given the proper value, in the manner which will be described, the fall of potential across any one of the coils is 0.1 volt so that as M is shifted from the zero point D the potential difference between M and D is increased 0.1 volt at each step. Likewise, when the current is in adjustment, the shifting of M' away from D increases by infinitesimal known fractions of a volt the difference of potential between M and M'.

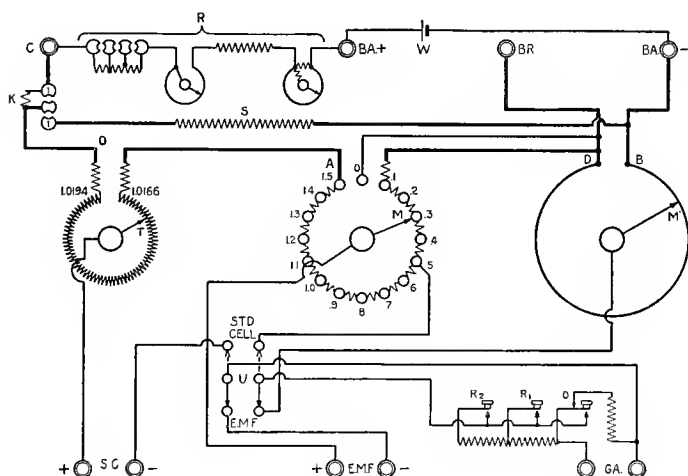


FIG. 22. WIRING OF THE LEEDS AND NORTHRUP POTENTIOMETER (TYPE K)

Now to adjust the potentiometer *current* so that the several resistances in the potentiometer circuit will produce the differences of potential in terms of which the instrument is calibrated, use is made of the Weston cell in the following manner. By means of a switch the unknown is thrown out and the Weston cell is thrown into circuit. One pole of the Weston cell circuit is fixed permanently. The other can be moved along a resistance at T constructed so that the dial indicates the value of the particular Weston cell in use. When so moved to agree with the particular cell in use, this contact at T is left in its position. Now the current

flowing from the battery W is adjusted by means of the rheostat R until the difference of potential between T and 0.5 balances the potential difference of the Weston cell as indicated by the cessation of current in the galvanometer Ga. The resistance $T - 0.5$ is such that the E. M. F. of the battery acting across this resistance will produce the desired potentiometer current. This current now acting across the several resistances furnishes the indicated potentials, i.e., a potential difference of 0.1 volt across each coil.

Another arrangement which employs the ordinary sets of resistances in common use is illustrated in figure 24.

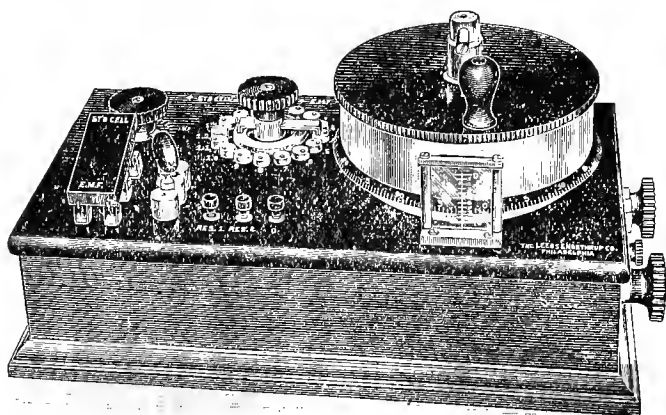


FIG. 23. THE LEEDS AND NORTHRUP POTENTIOMETER

A and B are duplicate sets of resistances placed in series with the battery S. If the current be kept uniform throughout this system the potential difference across the terminals of B can be varied in accordance with Ohm's law by plugging in or out resistance in B. But to keep the current constant while the resistance in B is changed a like resistance is added to the circuit at A when it is removed from B, and removed from A when it is added to B.

As mentioned before, the potential difference could be determined from the resistance in B and a measurement of the current but this is avoided by the direct application of a Weston cell of known potential. Assuming *constant current* a Weston cell replaces X and adjustment to the null point is made by altering the resistance in B. The unknown is then thrown into

circuit and adjustment of resistance again made to the null point. If E_w is the known E. M. F. of the Weston cell, E_x the potential of the measured cell, R_w the resistance in circuit when the Weston cell is in balance and R_c the resistance in circuit when the measured cell is in balance we have

$$C \text{ (constant)} = \frac{E_x}{R_c} = \frac{E_w}{R_w}$$

Whence

$$E_x = \frac{E_w R_c}{R_w}$$

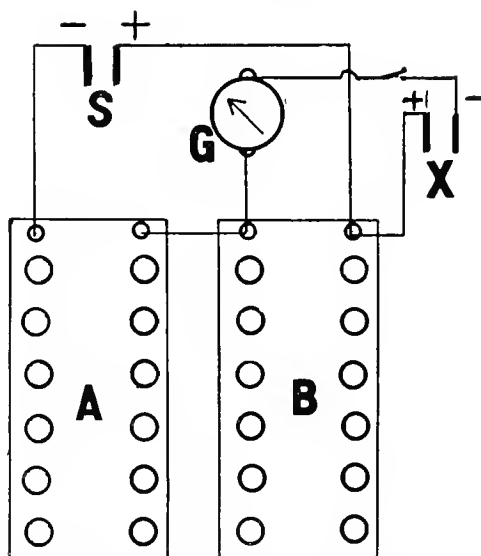


FIG. 24. ELEMENTARY RESISTANCE BOX POTENTIOMETER SYSTEM

The system is improved by providing means of regulating the potentiometer current till constant difference of potential is attained between terminals at which a Weston cell may be thrown into circuit. Then the resistances may be calibrated in volts.

It will be noted that in this arrangement every switch or plug contact is *in the potentiometer circuit*. A bad contact such as may be produced by failure to seat a plug firmly during the plugging in and out of resistance or by corrosion of a plug or dial contact will therefore seriously affect the accuracy of this potentiometer system. It requires constant care.

Lewis, Brighton and Sebastian (1917) used two decade resistance boxes of 9999 ohms each. With an external resistance the current was adjusted to exactly 0.0001 ampere. Thus each ohm indicated by the resistance boxes when balance was attained corresponded to 0.0001 volt. Their standard cell which gave at 25° 1.0181 volts was spanned across B (fig.24) and 182 ohms of the external resistance.

Another mode of using the simple system illustrated in figure 21 is the following. Instead of calibrating unit lengths along AB

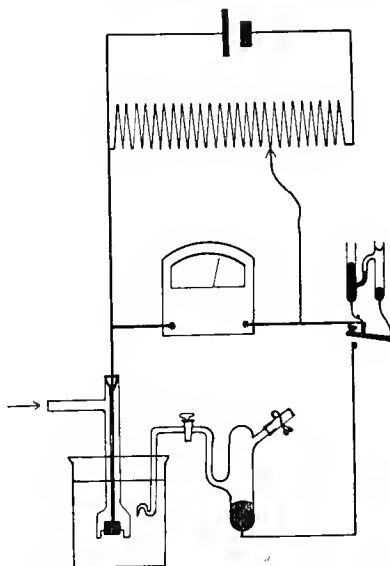


FIG. 25. VOLT METER POTENTIOMETER SYSTEM

by means of the Weston cell the contacts C and D carry the terminals of a volt meter. When balance is attained this volt meter shows directly the difference of potential between C and D, and therefore the E. M. F. of X.¹

¹ It is sometimes assumed that because the circuit of the system under measurement is placed in the *position* of a shunt on the potentiometer circuit that its resistance must be high in order that CD (fig. 21) may indicate correctly the potential difference. The fact that no current flows in this branch when balance obtains shows clearly that its resistance can have no effect on the accuracy of the indication. It has also been assumed that if CD is spanned by a voltmeter, the resistance of the voltmeter should be taken into consideration. But a voltmeter is *calibrated* to always indicate the potential difference between its terminals.

A diagram of such an arrangement is shown in figure 25. There is an apparent advantage in the fact that the Weston cell may be dispensed with and resistance values need not be known. There are however serious limitations to the precision of a voltmeter and in two cases which the author knows accuracy within the limited precision of the instruments was attained only after recalibration.

NULL POINT INSTRUMENTS

Referring to figure 21 and the accompanying text the reader will see that in the balancing of potential differences by the Poggen-dorf compensation method there is required a current indicating

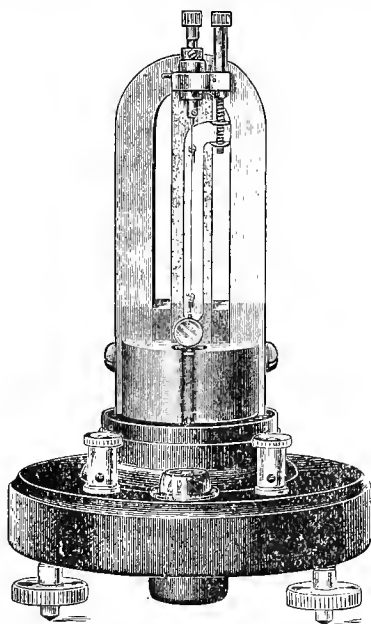


FIG. 26. A GALVANOMETER

instrument to determine the null point. Three such instruments will be briefly described, and some of their characteristics discussed later.

The galvanometer is a current indicating instrument, which, in the form most useful for the purpose at hand, consists of a coil of wire in the magnetic field of a strong permanent magnet. This

coil is connected into the circuit in which the presence or absence of current is to be detected. A current flowing through the turns of the suspended coil produces a magnetic field in its interaction with the field of the permanent magnet and tends to turn the coil so that it will embrace the maximum number of lines of force. The construction of galvanometers need not be discussed since it is a matter for instrument makers, but certain desirable qualities will be treated in a later section, together with the characteristics of other instruments.

Provision should be made for the mounting of a galvanometer where it will receive the least vibration. If the building is subjected to troublesome vibrations some sort of rubber support may be interposed between the galvanometer mounting and the wall bracket or suspension. Three tennis balls held in place by depressions in a block of wood on which the galvanometer is placed may help. In some instances the more elaborate Julius suspension such as those advertised may be necessary.

The capillary electrometer depends for its action upon the alteration of surface tension between mercury and sulfuric acid with alteration of the potential difference at the interface. A simple form suitable for that degree of precision which does not call for the advantages of a galvanometer is illustrated in figure 27.

Platinum contacts are sealed into two test tubes and the tubes are joined as illustrated by means of a capillary K of about 1 mm. diameter. In making the seals between capillary and tubes the capillary is first blown out at each end and can then be treated as a tube of ordinary dimensions in making a T joint. After a thorough cleaning the instrument is filled as illustrated with clean distilled mercury, sufficient mercury being poured into the left tube to bring the meniscus in the capillary near a convenient point. In the other tube is now placed pure sulfuric acid diluted 1 to 10. The air is forced out of the capillary with mercury until a sharp contact between mercury and acid occurs in the capillary. The instrument is now mounted before a microscope using as high power lenses as the radius of the glass capillary will permit. The definition of the mercury meniscus is brought out by cementing to the capillary with Canada balsam a cover glass as illustrated.

An important feature in the use of the capillary electrometer is its short circuiting between measurements. This is done by the

key shown in figure 27. Tapping down on the key breaks the short circuit and brings the terminals of the electrometer into circuit with the E. M. F. to be balanced. If the E. M. F. is out of balance the potential difference at the mercury-acid interface causes

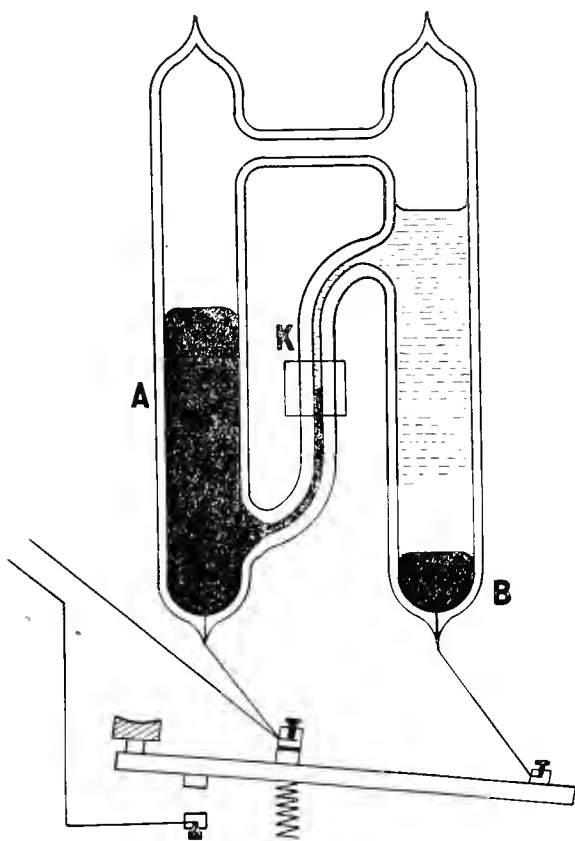


FIG. 27. DIAGRAM OF CAPILLARY ELECTROMETER AND KEY

the mercury to rise or fall in the capillary. Releasing the key short-circuits the terminals and allows the mercury to return to its normal position. Adjustment of the potentiometer is continued till no movement of the mercury can be detected. To establish a point of reference from which to judge the movement

of the mercury meniscus the microscope should contain the familiar micrometer disk at the diaphragm of the eye piece. In lieu of this an extremely fine drawn thread of glass or a spider web may be held at the diaphragm of the eye piece by touches of Canada balsam.

The quadrant electrometer is so little used as a null point instrument that its principle will not be described. It may be set up in the potentiometer arrangement with the needle charged from a grounded battery and the opposite quadrants connected as are the terminals of the capillary electrometer with a shortcircuiting key. Since the current drawn for its operation is only the amount required to charge the opposite quadrants at a very low potential difference, should the measured E. M. F. be unbalanced, and, at balance, zero potential difference, the quadrant electrometer might be of special value in the study of easily displaced electrode equilibria. The Dolezalek design has been developed until there has been attained a ruggedness together with a sensitivity that is encouraging. However, to attain the desired sensitivity with some of these instruments the negative electrostatic control must be raised to a value which renders the zero position of the needle unstable. This combined with the very long period at high sensitivity makes the instrument rather unsatisfactory for ordinary use.

Selection of null point indicators. In the selection of instruments for the measurement of the electromotive force of gas chains it is desirable that there should be a balancing of instrumental characteristics and the selection of those best adapted to the order of accuracy required. A null point instrument of low sensitivity may annul the value of a well-designed, expensive and accurate potentiometer; and a galvanometer of excessive sensitivity may be very disconcerting to use. The potentiometer system and the null point instrument should be adapted one to the other and to their relation to the system to be measured.

The several corrections which have to be found and applied to accurate measurements of hydrogen electrode potentials are matters of a millivolt or two and fractions thereof. Collectively they may amount to a value of the order of 5 millivolts. Whether or not such corrections are to be taken into account is a question whose answer may be considered to determine whether a rough

measuring system or an accurate one is to be used. For all "rough" measurements the capillary electrometer is a good null point instrument. It has a very high resistance which hinders the displacement of electrode equilibria at unbalance of a crude potentiometer system. It is easily constructed by anyone with a knowledge of the elements of glass blowing, and without particular care may be made sensitive to 0.001 volt.

For "accurate" measurements there is little use in making an elaborate capillary electrometer or in temporizing with poor galvanometers.

The apportionment of galvanometer characteristics is a complicated affair which must be left in the hands of instrument makers, but there are certain relations which should be fulfilled by an instrument to be used for the purpose at hand and general knowledge of these is quite necessary in selecting instruments from the wide and often confusing variety on the market.

Galvanometer sensitivities are expressed in various ways. Since ones attention is centred upon detecting potential differences the temptation is to ask for the galvanometer sensitivity in terms of microvolt sensitivity. There are two ways of expressing this which lead to different values. One is the deflection caused by a microvolt acting at the terminals of the galvanometer. The more useful value is the deflection caused by a microvolt acting through the external critical damping resistance. But in the last analysis the instrument is to be used for the detection of very small *currents* and these currents when allowed to flow through the galvanometer by the unbalancing of the circuit at a slight potential difference are determined by the total resistance of the galvanometer circuit. The instrument might be such that a microvolt at the terminals would cause a wide deflection, while, if forced to act through a large external resistance, this microvolt would leave the galvanometer "dead." For this reason it is best to know the sensitivity in terms of the *resistance* through which a unit voltage will cause a given deflection. This is the megohm sensitivity and is defined as "the number of megohms (million ohms) of resistance which must be placed in the galvanometer circuit in order that from an impressed E. M. F. of one volt there shall result a deflection of one millimeter" upon a scale one meter from the reflecting mirror (Leeds and Northrup catalogue

20, 1918). The numerical value of this megohm sensitivity also represents the microampere sensitivity if this is defined as the number of millimeters deflection caused by one microampere.

In hydrogen electrode measurements the resistance of the cells varies greatly with design (length and width of liquid conductors) and with the composition of the solutions used (e.g. saturated or M/10 KCl). Constricted, long tubes may raise the resistance of a chain so high as to annul the sensitivity of a galvanometer unless this has a high megohm sensitivity. Dr. Klopsteg (private communication) states that the resistance of the galvanometer coil ideally should be of about the same order of magnitude as that of the cell to be measured if maximum sensitivity is to be gained. Here however we enter complexities, since the arrangements by which high megohm sensitivity is attained affect other galvanometer characteristics. One of these, which is not essential but is desirable, is a short period. A short period facilitates the setting of a potentiometer. If the circuits are out of balance, as they generally are at the beginning of a measurement, the direction for readjustment may be inferred from the direction of galvanometer deflection without bringing the coil back each time to zero setting, but there comes a time when prompt return to zero setting is essential to make sure that slight resettings of the potentiometer are being made in the proper direction.

For a return of the coil to zero without oscillation it is necessary to have some sort of damping. This is generally a shunt across the galvanometer terminals, the so called critical damping resistance. This shunt permits a flow of current, when the main galvanometer circuit is opened, which is generated by the turning of the coil in the magnetic field. The magnetic field produced in the coil by this current interacting with the field of the permanent magnet tends to oppose the further swing of the coil. When the resistance of the shunt is so adjusted to the galvanometer characteristics that the swing progresses without undue delay to zero setting and there stops without oscillation, the galvanometer is said to be critically damped. Critical damping as applied to deflection on a closed circuit need not be considered when the galvanometer is used as a null point instrument. Since some of the best galvanometers are not supplied with a damping resistance the purchaser of an outfit for hydrogen electrode work should

take care to see that he includes the proper unit. Under-damped and over-damped instruments will prove troublesome.

These very brief considerations are presented merely as an aid in the selection of instruments. The manner in which desirable qualities are combined is a matter of considerable complexity but fortunately makers are coming to appreciate the very simple but important requirements for hydrogen electrode work and are prepared to furnish them. The galvanometer now in use by the author has the following characteristics; coil resistance 510 ohms, critical damping resistance 10,000 ohms, period 5.4 seconds, sensitivity 1973 megohms. It is not the ideal instrument for the hydrogen electrode system in use but is satisfactory. A shorter period is desirable and a higher coil resistance to correspond better with the average resistance of the order of one to two thousand ohms in some gas chains, would be desirable; but improvement in both of these directions at the same time may increase the expense of the instrument beyond the practical worth.

In using a galvanometer it is important to remember that while the E. M. F. of a cell is unbalanced its circuit should be left closed only long enough to show the *direction* of the galvanometer deflection. Otherwise current will flow in one direction or the other through the chain and tend to upset the electrode equilibrium. A mere tap on the key which closes the galvanometer circuit is sufficient till balance is obtained.

Of potentiometer characteristics little need be said for the reason that a choice will lie between instruments of reliable makers who have given proof of careful construction. Certain difficulties which enter into the construction of potentiometers for accurate thermo couple work are hardly significant for the order of accuracy required of hydrogen electrode work. The range from zero to 1.2 volts and the subdivisions 0.0001 volt do for measurements of ordinary accuracy. There should be a variable resistance to accommodate the variations in individual Weston cells of from 1.0175 to 1.0194 volts, and provision for quickly and easily interchanging Weston cell with measured E. M. F.

Several of the features of standard potentiometers may be eliminated without injury to their use for hydrogen electrode measurements and would reduce their cost. Steps in this direction are being taken by at least one manufacturer.

When rubber is used as the insulating material of instruments employed as potentiometers the rubber should not be left exposed to the light unduly. The action of the light not only injures the appearance of the rubber but also may cause the formation of conducting surface layers.

For very rough work the most attractive potentiometer system is that briefly mentioned on page 148 where use is made of a millivoltmeter. Figure 25 illustrates a set-up similar to that used by Hildebrand and others. In place of the electrometer there may be used one of the portable galvanometers which are now designed with high resistance coils for just such uses. The outstanding difficulty in the use of the millivoltmeter is its instrumental limitations. If the total range of the millivoltmeter is one volt, and the scale divisions are 0.01 volt spaced one millimeter apart errors of reading and calibration may easily be 0.005 volt or about 0.1 pH unit, and if the hydrogen electrode is joined with a saturated calomel electrode only that part of the scale between about 0.5 and 0.8 volts will be used in ordinary physiological studies.

Lists of three representative outfits are given in the appendix.

THE WESTON CELL

The construction of the Weston cell is illustrated in figure 28. The mercury in the left arm should be carefully purified (page 172) and the same material should be used for the preparation of the cadmium amalgam. This amalgam consists of 12.5 per cent by weight of electrolytic cadmium. The amalgam is formed by heating mercury over a steam bath and stirring in the cadmium. Any oxid formed may be strained off by pouring the molten amalgam through a test tube drawn out to a long capillary.

Cadmium sulfate may be recrystallized as described by Wolff and Waters (1907). Dissolve in excess of water at 70°C., filter, add excess of basic cadmium sulfate and a few cubic centimeters of hydrogen peroxid to oxidize ferrous iron, and heat several hours. Then filter, acidify slightly and evaporate to a small volume. Filter hot and wash the crystals with cold water. Recrystallize slowly from an initially unsaturated solution. The cadmium sulfate solution of a "normal" Weston cell is a solution saturated at whatever temperature the cell is used, and therefore the cell should

contain crystals of the sulfate. The ordinary unsaturated cell has a cadmium sulfate solution that is saturated at about 4°C .

In the study of Weston cells considerable attention has been paid to the quality of the mercurous sulfate. Perhaps the best and at the same time the most conveniently prepared material is that made electrolytically. Where the alternating current is available it is preferable to use it. A good average set of conditions is a sixty cycle alternating current sent through a 25 per cent sulfuric acid solution with a current density at the electrodes of 5 to 10 amperes per square decimeter. With either the alternating or direct current the apparatus described on page 135 is convenient.

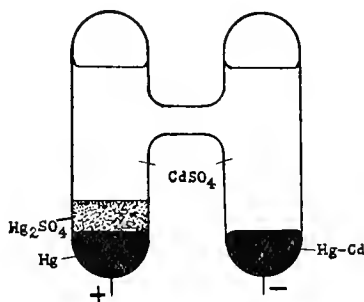


FIG. 28. DIAGRAM OF THE WESTON STANDARD CELL

In the Weston cell the lead-in wires of platinum should be amalgamated electrolytically by making a wire the cathode in a solution of pure mercurous nitrate in dilute nitric acid.

After filling the cell it may be sealed off in the blast flame or corked and sealed with wax.

Since the preparation of a good Weston cell is a matter of considerable detail, since such cells must be properly and carefully made to establish the true potential differences in a potentiometer system, and since reliable cells of certified values may be purchased at a reasonable price, it hardly pays the individual investigator to construct his own. It would, however, be a convenience if the materials could be purchased of the Bureau of Standards as was once proposed.

The portable Weston cells of commerce are for the most part of the unsaturated type. Instead of crystals of cadmium sulfate

being present at all temperatures the cadmium sulfate solution is saturated at about 4°C. This gives a cell with a much lower temperature coefficient than the normal Weston. It should, however, be remembered that there remain hysteresis and large if opposite temperature coefficients for the two arms and that therefore the cell should not be subjected to temperature fluctuations.

Commercial cells are standardized in terms of the international volt for the maintenance of the value of which the "normal" Weston cell is used.

As the result of cooperative measurements by the national standards laboratories of England, France, Germany and the United States the value 1.01830 international volts at 20°C. was assigned to the "normal" Weston cell. The United States Bureau of Standards maintains a group of these normal Weston cells whose mean value is taken as 1.0183 international volts and serves for the standardization of the commercial cells. It is important to note that this international agreement came into force January 1, 1911, and that prior to that time the values in force in different countries varied considerably.

The temperature coefficient of the "normal" Weston cell is given by Wolff (1908) as:

$$E_t = E_{20} - 0.000,040,75 (t - 20) - 0.000,000,944 (t - 20)^2 + 0.000,000,009,8 (t - 20)^3$$

By this formula the differences in volts from the 20° value are as follows:

TEMPERATURE	DIFFERENCE
°C.	
0	+0.000,359
5	+0.000,366
10	+0.000,304
15	+0.000,179
20	0.000,000
25	-0.000,226
30	-0.000,492
35	-0.000,791
40	-0.001,114

In other words a *normal* Weston cell should have its certified value corrected by addition of the above corrections when used at

temperatures other than 20°C. But an unsaturated Weston cell may for all ordinary purposes be considered as having no temperature coefficient and its certified value may therefore be used as given for all moderate variations from 20°C. The change in E. M. F. of the unsaturated type is less than 0.000,01 volt per degree.

STORAGE BATTERIES

The storage battery or accumulator is a convenient and reliable source of current for the potentiometer. Standard potentiometers are generally designed for use with a single cell which gives an E. M. F. of about two volts.

The more familiar cell to which our attention shall be confined consists of two series of lead plates immersed in a sulfuric acid solution of definite specific gravity. The plates of one series are connected to one pole of the cell and the plates of the other series are connected to the other pole. If the plates are covered with lead sulfate to begin with, a current passed through the cell will produce lead peroxid upon the plates by which the positive current enters and spongy lead upon the other plates. On charging, therefore, the plates in connection with the positive pole assume the brown color of the oxid while the plates in connection with the negative pole assume the slate color of the spongy metal. The poles should be distinctly marked so that one need not inspect the plates to distinguish the polarity but should the marks become obscured and the cell be a closed cell the polarity should be carefully tested with a voltmeter before attaching the charging current. In lieu of a voltmeter the polarity may be tested with a paper moistened with KI solution. On applying the terminals to the paper a brown stain is produced at the positive pole. The positive terminal of the charging circuit should be connected with the positive pole of the battery on charging otherwise the battery will be ruined.

The charging of a cell or a battery of cells may be done with the ordinary *direct current* lighting system if proper resistance be placed in series with the battery. A convenient resistance is made with electric light bulbs wired in parallel. A 16-candle power carbon filament on a 110-volt circuit allows about one half ampere to pass. If then the normal charging rate of the bat-

tery is 3 amperes a bank of 6 lamps in parallel will furnish the desired resistance. Ordinarily one can afford to charge at a rate lower than the normal rate specified by the manufacturer. On charging the voltage will rise rapidly to 2.35 volts where it will remain during the greater part of the period. When it rises to 2.5 volts the charging should be discontinued. It is when it has reached this voltage that the cell will "gas" vigorously. If a cell should fail to "gas" after a reasonable time it may have an internal short circuit due to warping of the plates or the scaling of conducting material. In searching for such a condition a wooden pry, never a metallic one, should be used. Careful handling and charging will generally prevent such short circuits.

In the discharging of a cell the sulfuric acid is converted to sulfate which is deposited. The result is the lowering of the specific gravity of the battery liquid. Thus the specific gravity of the liquid is highest when the battery is fully charged and lowers on discharging. If there be reason to suspect that the proper specific gravity is not being maintained it should be measured with a hydrometer. Fresh sulfuric acid may be added if one follows carefully the specifications given by the manufacturer of the cell. In making fresh solution only sulfuric acid free from metals other than lead, free from arsenic, and free from chloride and nitrate should be used. There will be a continuous loss of water from the battery liquid due to evaporation and gassing. This should be replaced by distilled water *during the recharging of the cell*.

In discharging a cell its voltage should not be allowed to fall below 1.8 volts. When a cell has reached this voltage it should be recharged immediately. If however the cell has been discharged to a lower voltage it should be recharged at half rate.

In using a storage cell to supply potentiometer current it is essential, that the highest stability in the current should be attained since the fundamental principle in the potentiometer involves the maintenance of constant current between the moment at which the Weston cell is balanced and the moment at which the measured E. M. F. is balanced. Steadiness of current is attained first by having a storage cell of sufficient capacity, and second by using it at the most favorable voltage. Capacity is attained by the number and size of the plates. A cell of 60 ampere hour capacity is sufficient for ordinary work. The current from a storage cell is

steadiest when the voltage has fallen to 2 volts. When a potentiometer system of sufficient resistance is used it is good practice to leave the cell in circuit, replacing it or recharging it of course when the voltage has fallen to 1.8 or 1.9 volts, and thus insure the attainment of a steady current when measurements are to be made.

In no case should a cell used for supplying potentiometer current be wired so that a throw of a switch will replace the discharging with the charging circuit. The danger of leakage from the high potential circuit is too great a risk for the slight convenience.

REFERENCES

Potentiometers

Bartell (1917), Bovie (1915), Hildebrand (1913), Leeds Northrup Catalogue 70, McClendon (1915), Wenner-Weibel (1914), White (1914).

Galvanometers

Leeds-Northrup Company Catalogue 20 (1918), White (1906).

Capillary electrometer

Boley (1902), Lippmann, G. (1875), Smith (1900) (1903).

Quadrant electrometer

Beattie (1910-12), Compton-Compton (1919), Dolezalek (1906).

Weston standard cell

Bureau Standards Circular 60, Report to International Committee (1912)
Wolff (1908), Wolff-Waters (1907), Hulett (1906).

International volt

Dellinger (1916), Bureau Standards Circulars Nos. 29, 60.

CHAPTER XIII

HYDROGEN GENERATORS, WIRING, SHIELDING, TEMPERATURE CONTROL, PURIFICATION OF MERCURY

Hydrogen generators. When there is no particular reason for attaining equilibrium rapidly at the electrode a moderate supply of hydrogen will do. When, however, speed is essential, or when there are used those immersion electrodes which are not well guarded against access of atmospheric oxygen an abundant supply of hydrogen is essential. Indeed it may be said that one of the most frequent faults of the cruder equipments is the failure to provide an adequate supply of pure hydrogen or the failure to use generously the available supply.

Frequently hydrogen for the hydrogen electrode is generated from zinc and sulfuric acid. Care should be taken to displace all oxygen from generator and to free the hydrogen from impurities, especially arsenic.

Compressed hydrogen of high purity is now on the market. It has been found satisfactory for hydrogen electrode work by Cullen (1917) and by Fales and Vosburgh (1918). The latter authors pass this hydrogen through alkaline permanganate, alkaline pyrogallate, water, cotton wool, and then a solution similar to that contained in the electrode vessel. Cullen passed this tank hydrogen through solutions of HgCl_2 , and KMnO_4 , pyrogallol, dilute sulfuric acid and water. In the use of alkaline pyrogallol it will be remembered that, unless the solution is carefully prepared, CO may be evolved. Chemical hydrogen generators with their accompanying wash bottles generally contain much free gas space and frequently several dead spaces. With occasional use, therefore, it is essential that the generator be run for a considerable time to displace the air which may have diffused into these spaces.

The compressed hydrogen should be especially valuable for immersion electrodes such as that of Hildebrand (see page 133) where there is required an abundant supply for occasional use in titrations. One should be on guard against tanks which have been used for other gases.

Electrolytic generators have been most frequently employed. The generator shown in figure 29 is constructed from an ordinary museum jar. The glass cover may be perforated by drilling with a metal tube fed with carborundum and glycerine. The electrolyte is 10 per cent sodium hydroxid and the electrodes nickel. To remove the spatter of electrolyte the gas passes over the layer of concentrated sulfuric acid shown in the figure, and then, to

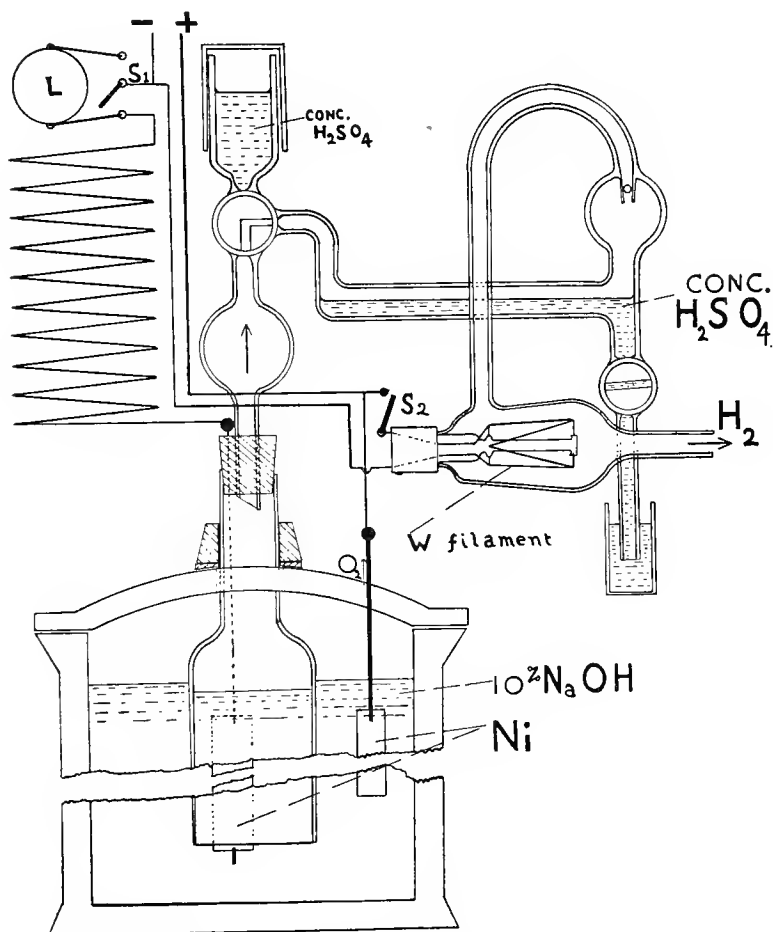


FIG. 29. AN ELECTROLYTIC HYDROGEN GENERATOR

burn out residual traces of oxygen, the gas passes through an ordinary tungsten filament lamp. In place of this there may be used the heated platinum wire described by Lewis, Brighton and Sebastian or a tube of platinized asbestos heated in a small electric furnace. In the author's design shown in figure 29 the wiring is so arranged that the generator while in use carries about 4.5 amperes. When the generator is not in use the switch S_1 is turned to throw into series a lamp. The generator then evolves only enough hydrogen to keep flushed out. In order to save the combustion lamp it is thrown out of circuit by S_2 when the generator is not in use. Such a generator has been run continuously for months at a time.

Since rubber connections are often used in the equipment for hydrogen electrode work it may be of interest to note the following relative rates of diffusion of gases through rubber.

<i>Gas</i>	<i>Rate</i>
Nitrogen.....	1.00
Air.....	1.15
Oxygen.....	2.56
Hydrogen.....	5.50
Carbon dioxide.....	13.57

Wiring. Whenever a set-up is to be made more than an improvisation it pays to make a good job of the wiring. A poor connection may be a source of endless trouble and unsystematized wiring may lead to confusion in the comparison of calomel electrodes and the application of corrections of wrong sign.

Soldered connections or stout binding posts that permit strong pressure without cutting of the wire are preferable to any other form of contact. If for any reason mercury contacts are used they had best be through platinum soldered to the copper lead. Copper wires led into mercury should not take the form of a siphon else some months after installation it may be found that the mercury has been siphoned off.

Thermo electromotive forces are seldom large enough to affect measurements of the order of accuracy with which we are now concerned if care be taken to make contacts so far as possible between copper and copper at points subject to fluctuations in temperature.

A generous use of copper knife switches, although it may entail electrical capacity undesired in some instances, can be made to contribute to the ease and certainty of check measurements. For instance if there be a battery of hydrogen electrodes and a set of calomel electrodes, wires may be led from each to a centre connection of single-pole, double-throw switches as shown in figure 30. All the upper connections of these switches are connected to the

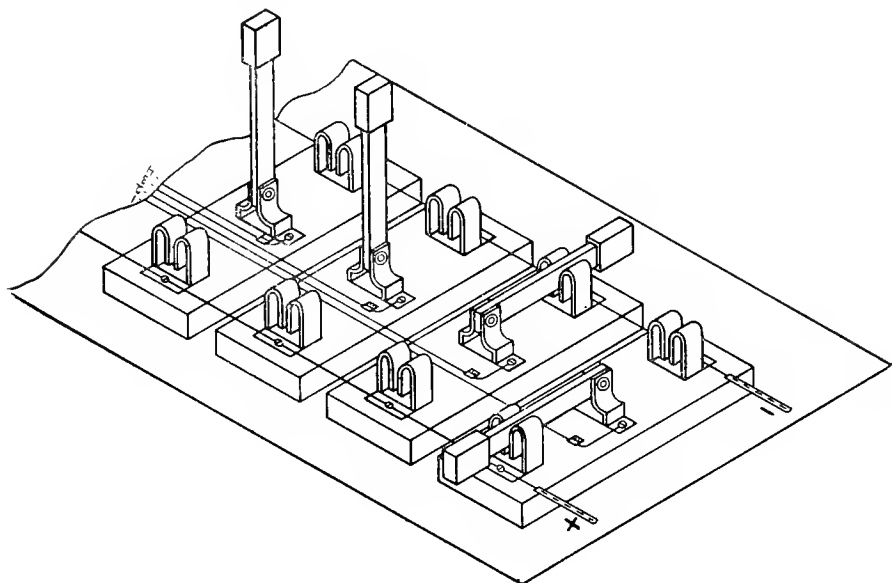


FIG. 30. SWITCHES FOR CONNECTING HALF-CELLS WITH POTENTIOMETER

+ pole of the potentiometer's E. M. F. circuit, and all the lower connections to the — pole. By observing the rule that no two switches shall be closed in the same direction, short-circuiting of combinations is avoided. The position of a switch shows at once the sign of its electrode in relation to any other that may be put in liquid junction. This is a great convenience in comparing calomel electrodes where one half-cell may be positive to another and negative to a third. Such a bank of single pole switches permits the comparison of any electrode with any other when liquid junction is established; and, if a leak occur in the electrical sys-

tem the ability to connect one wire at a time with the potentiometer and galvanometer often helps in the tracing of the leak.

Shielding Electrical leaks from surrounding high potential circuits are sometimes strangely absent from the most crude systems and sometimes persistently disconcerting if there is not efficient shielding. The principle of shielding is based on the following considerations. If between two supposedly well-insulated points on a light or heating circuit, or between one point of such a circuit and a grounding such as a water or drain pipe, there is a slight flow of current, the electrical charges will distribute themselves over the surface films of moisture on wood and glass ware. At two points between which there is a difference of potential the wires of the measured or measuring system may pick up the difference of potential to the detriment of the measurement. If however *all* supports of the measured and measuring systems lie on a good conductor such as a sheet of metal, the electrical leakage from without will distribute itself over an *equipotential* surface and no differences of potential can be picked up. To shield efficiently, then, it is necessary that *all* parts of the system be mounted upon metal that can be brought into good conducting contact. In many instances the complications of hydrogen electrode apparatus and especially the separation of potentiometer from temperature bath make a simple shielding impracticable. Care must then be taken that all of the separate parts are well connected. Tinfoil winding of wire in contact with unshielded points can be soldered to stout wires for connection to other parts by dropping hot solder on the well-cleaned juncture.

Shielding should not be considered as in any way taking the place of good insulation of the constituent parts of the measured or measuring systems.

For further details in regard to shielding see W. P. White (1914).

Temperature control is a matter where individual preference holds sway. There are almost as many modifications of various types of regulators as there are workers. Even in the case of electrical measurements where orthodoxy interdicts the use of a water bath it has been said (Fales and Vosburgh) that it can be made to give satisfaction.

Yet there are a few who may actually make use of a few words of suggestion regarding temperature control for hydrogen electrode work.

As a rule the water bath is not used because of the difficulty of preventing electrical leakage. Some special grades of kerosene are sold to replace the water of an ordinary liquid bath but for most purposes ordinary kerosene does very well. A liquid bath has the advantage that the relatively high specific heat of the liquid facilitates heat exchange and brings material rapidly to the controlled temperature, but compared with an air bath it has the disadvantage that stopcocks must be brought up out of the liquid to prevent the seepage of the oil. The advantage of the high specific heat of a liquid is sometimes falsely applied as when the constancy of a liquid bath is considered to be a great advantage over the more inconstant air bath. The lower the specific heat of the fluid the less effect will variation in the temperature of that fluid have upon material which it is desired to keep at constant temperature. For this reason a well stirred air bath whose temperature may oscillate about a well-controlled mean may actually maintain a steadier temperature in the material under observation than does a liquid bath which itself is more constant. It is the temperature of the material under observation and not the temperature of the bath which is of prime interest.

An air bath can be made to give very good temperature control and since it is more cleanly than an oil bath and permits directness and simplicity in the design of apparatus a brief description of one form used by the writer for some years may be of interest.

A schematic longitudinal section illustrating the main features is shown in figure 31.

The walls of the box are lined with cork board finished off on the interior with "compo board." The front is a hinged door constructed like the rest of the box but provided with a double glass window and three 4-inch hand holes through which apparatus can be reached. On the interior are mounted the two shelves A and B extending from the front to the back wall and providing two flues for the air currents generated by the centrifugal fan F. This fan is a number 0 Sirocco fan manufactured by The American Blower Company, demounted from its casing, and mounted in the bearing illustrated so that it can be run by a motor outside the air bath. The currents from this fan on returning to the working chamber are broken into parallel lines of flow by means of the baffle plates at E which are simply strips of tin arranged as are

the cardboard strips in an egg box. The heating of the air is done by bare nichrome wire no. 30 B. & S. gauge strung between two rings of asbestos board which fit over the fan at H. The air is thus heated at its position of highest velocity. The electrical current in this heating coil can be adjusted with the weather so that the time during which the regulator leaves the heat on is about as long as the time during which the regulator leaves the heat off. In

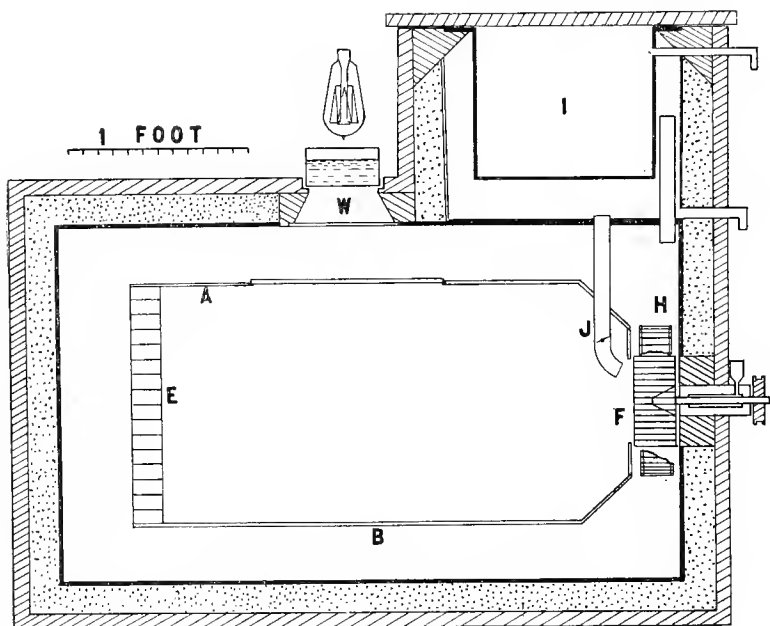


FIG. 31. CROSS SECTION OF AN AIR BATH

other words adjustment is made so that the heating and cooling curves have about the same slope, or so that the heating balances the loss of heat through the walls.

When the room temperature is not low enough to provide the necessary cooling the box I is filled with ice water. Surrounding this is an air chamber into which air is forced from the high pressure side of the fan. The cooled air then flows back through the pipe J to be delivered into the throat of the fan. J should be provided with a damper which can easily be reached and adjusted.

To lessen danger of electrical leakage over damp surfaces the air is kept dry by a pan of calcium chlorid.

A double window at W over which is hung an electric light provides illumination of the interior. A solution of a nickel salt is placed at this window to absorb the heat from the lamp.

Such a box has been held for a period of eight hours with no change which could be detected by means of a tapped Beckmann thermometer and with momentary fluctuations of 0.003 as determined with a thermo element. The average operation is a temperature control within $\pm 0.03^\circ$ with occasional unexplained variations which may reach 0.1° . Because of the slowness with which air brings material to its temperature the aid bath is continuously kept in operation.

Given efficient stirring and a considerate regulation of the current used in heating, accurate temperature control reduces to the careful construction of the regulator. For an air bath the ideal regulator should respond instantaneously. This implies rapid heat conduction. Regulators which provide this by having a metal container have been described but glass will ordinarily be used. At all events there are two simple principles of regulator construction the neglect of which may cause trouble or decrease sensitivity and attention to which improves greatly almost any type. The first is the protection of the mercury contact from the corroding effect of oxygen. The second is the elimination of platinum contacts which mercury will sooner or later "wet" and the substitution of an iron, nickel or nichrome wire contact.

After trials of various designs the author has adopted the two forms of regulator heads shown in figure 32.

For precise control at an inaccurately adjusted temperature form A is used. The platinum lead-in wire P is fused to the nichrome wire N. After filling the instrument with mercury dry hydrogen is flushed through the head by way of the side tubes. These are then sealed off and serve as reservoirs for excess mercury. Adjustment is made by slightly overheating the body of the mercury, breaking off the capillary column by a tap of the hand and storing the detached portion in one of the side tubes. Such an adjustment is often troublesome when regulation at a particular temperature is desired but once the adjustment is made it is permanent, provided the contact wire is ground down to a

fine thread so that it will not fill the capillary enough to cause the mercury thread to part on occasions of overheating.

Form B permits delicate adjustment of the contact by means of the screw S but it requires skill to make such a head properly. The nichrome wire must fit very closely in the capillary R to pre-

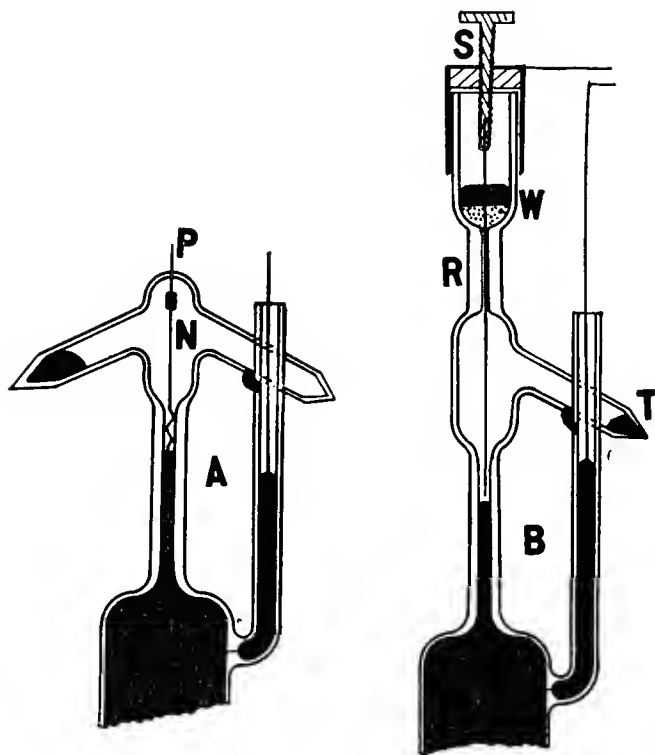


FIG. 32. THERMO-REGULATOR HEADS

vent the wax and mercury seal at W from creeping downward. Such a close fit implies very careful glass blowing to maintain a straight and unconstricted capillary. With the contact wire in place and the proper amount of mercury in the apparatus hydrogen is run in at T escaping through R. Then a bit of bees wax is melted about W and at the moment it hardens the hydrogen supply is shut off, T is sealed, and then the wax is covered with a *shallow* layer of mercury.

For an air bath it is best to seal such regulator heads to a grid of tubes.

The permanency of regulators of such design when properly made is a great asset and well worth care in preparation. Regulators of each of these types have been in continuous operation for years without serious trouble. One of type A survived a severe laboratory fire and after readjustment is still in operation.

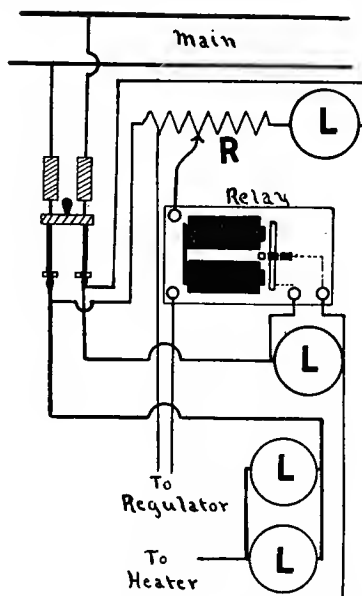


FIG. 33. WIRING FOR TEMPERATURE CONTROL

Filling such regulators with mercury can be done most easily by first evacuating the vessel under some one of the various high vacuum pumps and then letting the mercury in slowly through one of the side arms drawn to a fine point which is broken under mercury.

A description of methods of purifying mercury will be found on page 172.

For electrical control of temperature the following scheme of wiring has proved satisfactory (fig. 33).

Lamps which are neat, convenient, replacable forms of resistance obtainable in variety and which indicate whether or not current

is flowing are shown in figure 33 by L. R is a resistance formed by a few turns of number 30 nichrome wire on pyrex glass, porcelain or asbestos board. By shifting the brass contact clamp along this resistance the proper amount of current to operate the relay may be found by trial. Too strong a current is to be avoided. A sharp, positive action of the relay should be provided against the day when the relay contact may become clogged with dust. To reduce sparking at the regulator and at the relay contacts, inductive coils in the wiring should be avoided. Spanning the spark gaps with properly adjusted condensers made of alternate layers of tin foil and paraffine paper may eliminate most of the sparking, if the proper capacity be used. For air regulation it is essential that the heater be of bare wire so that it cools the moment the current is turned off. Furthermore it is essential to reduce the current till the heating rate is close to the cooling rate of the air bath. For such control of the heating current there are inserted in series with the heater two lamp sockets in parallel permitting either the insertion of a fuse, one lamp or two lamps of various sizes. The other lamp shown in the heating circuit reduces sparking at the relay.

For relay contacts the tungsten contacts used in gas engines are very good.

Purification of mercury. Pure mercury is essential for many purposes in hydrogen electrode work,—for the calomel and the mercury of calomel electrodes, for Weston cells should these be “home made,” for thermo regulators and for the capillary electrometer.

The more commonly practiced methods of purification make use of the wide difference between mercury and its more troublesome impurities in what may be descriptively put as the “electrolytic solution tension.” Exposed to any solution which tends to dissolve base metals the mercury will give up its basic impurities before it goes into solution itself, provided of course the reaction is not too violent for the holding of equilibrium conditions.

The most commonly used solvent for this purpose is dilute nitric acid although a variety of other solutions such as that of ferric iron may be used.

To make such operations efficient it is necessary to expose as large a surface as possible to the solution. Therefore the mercury

is sometimes sprayed into a long column of solution which is supported by a narrow U-tube of mercury. The mercury as it collects in this U-tube separates from the solution and runs out into a receiver. To insure good separation the collecting tube should be widened where the mercury collects but this widening should not be so large as to prevent circulation of all the mercury. A piece of very fine meshed silk tied over the widened tip of a funnel makes a fine spray if the silk be kept under the liquid. This simple device can be made free from dead spaces so that all the mercury will pass through successive treatments. It is more difficult to eliminate these dead spaces in elaborate apparatus; but such apparatus, in which use is made of an air lift for circulating the mercury, makes practicable a large number of treatments. A combination of the air lift with other processes and a review of similar methods has been described by Patten and Mains (1917).

Hulett's (1905, 1911) method for the purification of mercury consists in distilling the mercury under diminished pressure in a current of air, the air oxidizing the base metals. Any of these oxids which are carried over are filtered from the mercury by passing it through a series of perforated filter papers or long fine capillaries. A convenient still for the purpose is made as follows. Fuse to the neck of a Pyrex Kjeldahl flask a tube about a foot long which raises out of the heat of the furnace the stopper that carries the capillary air-feed. Into the neck of the flask fuse by a T-joint seal a half inch tube and bend this slightly upward for a length of 6 inches so that spattered mercury may run back. To the end of this 6-inch length join the condensing tube, which is simply an air condenser made of a 3-foot length of narrow tubing bent zig-zag. Pass the end of this through the stopper of a distillation flask and attach suction to the side tube of this flask. The mercury in the Kjeldahl flask may be heated by a gas flame or an electric furnace. For a 220 volt D. C. circuit 40 feet of no. 26 nichrome wire wound around a thin asbestos covering of a tin can makes a good improvised heating unit if well insulated with asbestos or alundum cement. A little of this cement applied between the turns of wire after winding will keep the wire in place after the expansion by the heat.

In the construction of such stills it is best to avoid soft glass because of the danger of collapse on accidental over heating. Hostetter and Sosman describe a quartz still.

Both the air current, that is delivered under the surface of the mercury by means of a capillary tube, and the heating should be regulated so that distillation takes place smoothly.

Since it is very difficult to remove the last traces of oxid from mercury prepared by Hulett's distillation the author always makes a final distillation in vacuo at low temperature. An old but good form of vacuum still is easily constructed by dropping from the ends of an inclined tube two capillary tubes somewhat over barometric length. One of these is turned up to join a mercury reservoir, the other, the condenser and delivery tube, is turned up about 4 inches to prevent loss of the mercury column with changes in external pressure. The apparatus is filled with mercury by suction while it is inclined to the vertical. Releasing the suction and bringing the still to the vertical leaves the mercury in the still chamber supported by a column of mercury resting on atmospheric pressure and protected by the column in the capillary condenser. The heating unit is wire wound over asbestos. The heat should be regulated by a rheostat till the mercury distills very slowly. By having the mercury condense in a capillary the still becomes self-pumping.

Perhaps few of us who work with mercury have a proper regard for the real sources of danger to health. The vapor pressure of mercury at laboratory temperatures is not to be feared, but emulsification with the dust of the floor may subdivide the mercury until it can float in the air as a distinct menace. Its handling with fingers greasy with stop cock lubricant is also to be avoided on account of the ease with which the skin is penetrated by mercury emulsions.

CHAPTER XIV

THE RELATION OF HYDROGEN ELECTRODE POTENTIALS TO REDUCTION POTENTIALS

As indicated in Chapter IX the hydrogen electrode is but a special case of a general relation for the potential difference between a metal and a solution. The hydrogen electrode is constructed of a noble metal laden with hydrogen, and it may be asked what relation it bears to those electrodes which consist of the noble metal alone and which are used to determine the so-called oxidation-reduction potentials of solutions such as mixtures of ferrous and ferric iron.

If a platinum or gold electrode be placed in a mixture of ferrous and ferric sulfate there will almost immediately be assumed a stable potential difference which is determined by the *ratio* of the ferrous to the ferric *ions*. The relation which is found to hold is given by the equation:

$$E = C - \frac{RT}{nF} \ln \frac{[\text{Ferro}]}{[\text{Ferri}]}$$

where E is the observed potential difference between the electrode and a standard such as the normal hydrogen electrode, C is a constant characteristic of this particular oxidation reduction equilibrium and equal to E when the ratio $\frac{[\text{Ferro}]}{[\text{Ferri}]}$ is unity, R, T, n and F have their customary significance, and [Ferro] and [Ferri] represent concentrations of the ferrous and the ferric ions respectively. This equation will be referred to later as Peters' equation. Its general form is:

$$E = C - \frac{RT}{nF} \ln \frac{[\text{reduction product}]}{[\text{oxidation product}]} \quad (31)$$

If we plot E on the abscissa and the ratio

$$\frac{\text{concentration of reduction product}}{\text{concentration of oxidation product}}$$

on the ordinate, we obtain a set of curves each of which is identical in form for like values of n , and each of which has its position along the E axis determined by C . A series of such curves is shown in figure 34.

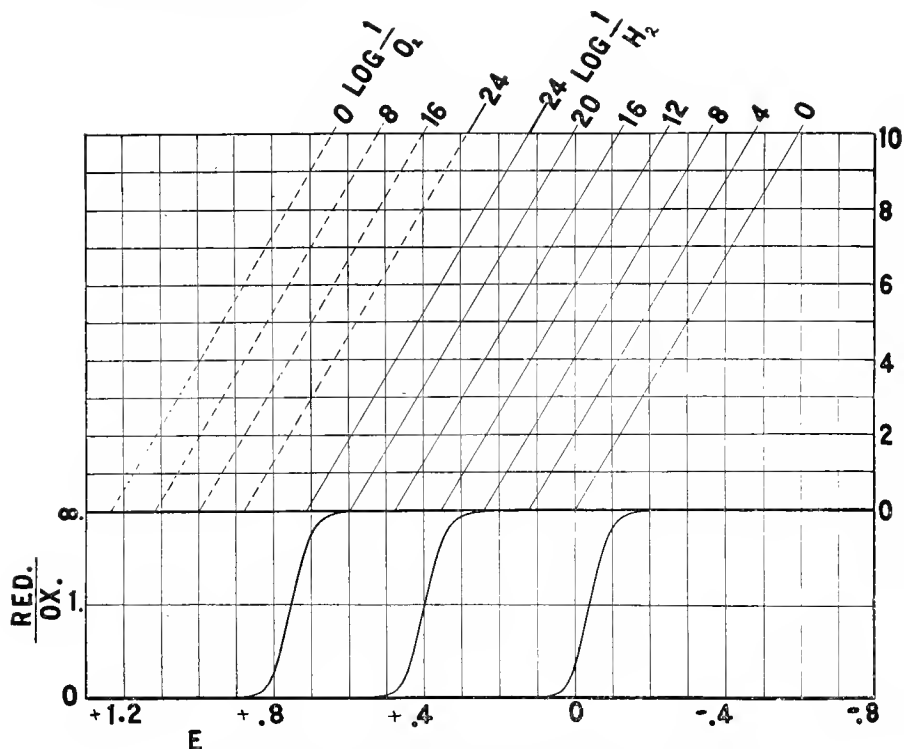


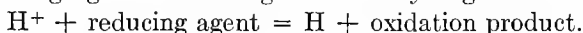
FIG. 34. DIAGRAM OF OXIDATION-REDUCTION RELATIONS

Below: Typical curves relating electrode potentials to the ratio reduction product.
oxidation product

Above: Potentials (abscissas) of electrodes at different pH values (ordinates) when the electrode is in equilibrium with oxidation-reduction equilibria producing hydrogen or oxygen pressures (diagonals).

Now it is known that certain reducing agents are so active that they evolve hydrogen from aqueous solutions. In such a solution an electrode would become charged with hydrogen and would

conduct itself much like a hydrogen electrode. If we represent the reducing agent interacting with the hydrogen ions we have:



At this point attention should be called to the fact that we are here speaking of oxidation-reduction in its electrolytic sense, and that oxygen may be no more concerned than it is when the charge on the ferrous ion is changed to that of the ferric ion by the action of some such "oxidizing agent" as chlorine.

If equilibrium is established for the above reaction

$$\frac{[\text{H}^+] \times [\text{reduction product}]}{[\text{H}] \times [\text{oxidation product}]} = K$$

or

$$K \frac{[\text{H}]}{[\text{H}^+]} = \frac{[\text{reduction product}]}{[\text{oxidation product}]}$$

Substituting

$$K \frac{[\text{H}]}{[\text{H}^+]} \text{ for the ratio } \frac{[\text{reduction product}]}{[\text{oxidation product}]}$$

in Peters' equation (31) we have

$$E = C - \frac{RT}{nF} \ln K \frac{[\text{H}]}{[\text{H}^+]}$$

Since the atomic hydrogen, H, bears a definite relation to the molecular hydrogen through the equilibrium

$$\frac{[\text{H}]^2}{[\text{H}_2]} = K_H \quad \text{or} \quad [\text{H}] = \sqrt{K_H \text{H}_2}$$

we may substitute and collect constants under another constant K' and so obtain

$$E = C - \frac{RT}{nF} \ln K' \frac{\sqrt{[\text{H}_2}]}{[\text{H}^+]}$$

or taking K' from under the log sign and combining it with C we have

$$E = C' - \frac{RT}{F} \ln \frac{\sqrt{[\text{H}_2}]}{[\text{H}^+]}. \quad (32)$$

Compare this with the general relation for the hydrogen electrode

$$E = C - \frac{RT}{F} \ln \frac{\sqrt{[\text{H}_2}]}{[\text{H}^+]}$$

Here $C = 0$, *by definition* of the potential difference at the normal hydrogen electrode. Hence, if potential differences are referred to this basis, $C = C'$. E of any oxidation reduction equilibrium may thus be expressed *in terms of* the E of a hydrogen electrode.

Physically this means that we assume an equilibrium between hydrogen and hydrogen ions in harmony with the equilibrium between the oxidation and reduction products in a solution, and that wherever we have such products present the electrode is charged with hydrogen at some definite pressure. If there are present oxidizing agents the hydrogen that may be in the electrode is withdrawn until its pressure is reduced to a value in harmony with the oxidation-reduction equilibrium in the solution. If a constant supply of hydrogen is afforded, as it is supposed to be in the customary use of the hydrogen electrode, no true equilibrium can be reached till this hydrogen has reduced the substances in solution to a point where they will support one atmosphere pressure of hydrogen.

Let the subject be followed further with the aid of figure 34. In this figure there are shown some typical curves for the relation of potential to ratio of reduction—oxidation products, and just above this is a scheme for showing the relation of such potentials to hydrogen potentials. Electrode potential differences in volts referred to the normal hydrogen electrode are shown on the abscissa. Zero potential is the potential difference at a hydrogen electrode in a solution normal with respect to hydrogen ions ($\text{pH} = 0$) and under one atmosphere pressure of hydrogen. If such an electrode is held under one atmosphere of hydrogen and the pH of the solution is varied, the potential differences will fall along the diagonal line farthest to the right. In other words true hydrogen electrode values all fall on this line. Conversely if we have a solution of such an intense reducing action that hydrogen at one atmosphere pressure is evolved and equilibrium is established, the potential difference shown at a platinized electrode will fall on this line at a point determined by the pH of the solution.

In a similar way we can calculate the potentials of a hydrogen electrode under one ten-thousandth atmosphere of hydrogen at all pH values. The line showing these potentials is marked $\log \frac{1}{\text{H}_2} = 4$. An oxidation-reduction equilibrium of such intensity that it will

charge a platinum electrode at $\frac{1}{1000}$ atmosphere pressure of hydrogen should show a potential difference at a point along the $\log \frac{I}{H_2} = 4$ line determined by the pH of the solution, e.g., about 0 at pH = 2 and about -0.3 at pH = 7. In a similar way the potentials, hydrogen pressures and pH values may be related under any condition. A hydrogen electrode then may be considered as a special case of an oxidation-reduction electrode where the solution is so far reduced that it will support one atmosphere pressure of hydrogen; while an oxidation-reduction electrode may be regarded as a hydrogen electrode under a reduced hydrogen pressure which is in equilibrium with the oxidation-reduction products in the solution.

Another interesting hypothetical relation may be illustrated by means of figure 34. There are certain theoretical reasons for believing that an oxy-hydrogen gas cell, i.e., a cell composed of a hydrogen and an oxygen electrode, each under one atmosphere pressure of the respective gases, should show an E. M. F. of 1.23 volts at all pH values. The actual conduct of the oxygen electrode is not at all well understood but, assuming that we have an "ideal oxygen electrode," it is evident that if the potential is always 1.23 volts more positive than a hydrogen electrode at the same pH the diagonal line at the extreme left of figure 34 represents the relation between the potential of an oxygen electrode and pH when the oxygen pressure is one atmosphere. If the potential varies as the logarithm of the fourth root of the oxygen pressure as the potential of a hydrogen electrode varies with the logarithm of the square root of the hydrogen pressure, we can lay off (as dotted lines in figure 34) the diagonals showing the pressures of oxygen about an electrode induced by an oxidizing agent at any pH and at any given potential.

Such relations, which have an undoubted physical significance in certain instances, become mere mathematical extrapolations or "calculation values" in other instances.

For example, in an equimolecular mixture of ferrous and ferric sulfate in an acid solution of hydrogen ion concentration pH = 1, an indifferent electrode (platinum) will have a potential of about 0.75 volts more positive than a normal hydrogen electrode. Let us consider this to be the difference of potential between a hydro-

gen electrode at $\text{pH} = 1$ and a normal hydrogen electrode. Let us calculate then the hydrogen pressure at 25°C . from the equation:

$$0.75 = -0.0599 \log \frac{\sqrt{[\text{H}_2]}}{0.1}$$

We find the hydrogen pressure to be about 10^{-27} atmospheres. At one atmosphere pressure a gram mol of hydrogen occupies about 22 litres and contains about 6×10^{23} molecules. If the pressure is reduced to 6×10^{-23} atmospheres there would be but one molecule of hydrogen in 22 litres. If reduced to 10^{-27} atmospheres there would be but one molecule in about 37,000 litres. To assume any physical significance in such values is, of course ridiculous.

It is only by courtesy then that an electrode in a mixture of ferrous and ferric iron at $\text{pH} 1$ can be considered as a hydrogen electrode.

This is but another instance of the physically impossible values encountered when *restricted points of view and restricted methods of expressing relations* are applied to electrode potential differences. One or two of these instances will be given to illustrate the fact that our present equations are incomplete in that they tell us little or nothing about the mechanisms at electrodes (see Langmuir 1916, also Smits and Aten 1916).

Lehfeldt (1899) says of the so-called solution pressures postulated by Nernst and briefly discussed in Chapter IX:

.	we have Zinc.....	9.9×10^{18}
	Nickel.....	1.3×10^9
	Palladium.....	1.5×10^{-36}

The first of them is startlingly large. The third is so small as to involve the rejection of the entire molecular theory of fluids.

Lehfeldt then shows that, in order to permit at the electrode the pressure indicated above for palladium, the solution would have to be so dilute as to contain but one or two ions of palladium in a space the size of the earth. No stable equilibrium could be measured under such a circumstance. On the other hand Lehfeldt calculates that to produce the high pressure indicated for zinc "1.27 grams of the metal would have to pass into the ionic form per square centimeter, which is obviously not the case."

There is thus very good reason to refrain from attributing a limited and sometimes obviously untrue physical significance to the integration constant in the fundamental equation for electrode potentials (see page 101).

Another aspect of the matter was emphasized in a lively discussion between Haber, Danneel, Bodländer and Abegg in *Zeitschrift für Elektrochemie*, 1904. Haber points out that, if the well established relation between silver ion concentration and the potential difference between a silver electrode and a solution containing silver ions be extrapolated to include the conditions found in a silver cyanide solution, the indicated concentration of the silver ion will be so low as to have no physical significance. Haber mentions the experiment of Bodländer and Eberlein where the potential and the quantity of solution were such that there was present at any moment less than one discrete silver ion. The greater part of the discussion centred upon the resolution of the equilibrium constant into a ratio of rates of reaction, and upon the conclusion that, if the silver ion in the cyanide solution has a concentration of the order of magnitude calculated, it must react with a speed greater than that of light or else that the known reactions of silver in cyanide solutions must take place partly with the silver complexes and not wholly with the silver ions. However we are now more directly concerned with another aspect of this interesting situation. The potentials observed in silver cyanide solutions are well defined. We may choose to extend to such solutions the relation between the potential of a silver electrode and silver ion concentration. When we do we find that the silver ion concentration by itself cannot account for the well-defined potential. How then is the stable and reproducible potential supported?

None of these discussions affect in any serious way those relations for concentration chains which are founded upon thermodynamic reasoning provided it be remembered that the thermodynamic reasoning alone does not furnish any conception of the physical mechanisms of a process. The points mentioned do however make it evident that values sometimes used are mere "calculation numbers" employed in a region of extrapolation where they have no real physical significance. The inevitable conclusion is that our equations are insufficiently generalized. This

may be illustrated if we return to a consideration of oxidation-reduction electrodes.

As already shown the potential of an indifferent electrode in a mixture of ferrous and ferric sulfate is a stable potential which cannot be considered as if it were a hydrogen electrode potential under greatly reduced hydrogen pressure. We must regard the platinum as a direct medium for the exchange of electrons between ferrous and ferric ions. Even to consider it as a hydrogen electrode potential for the sake of unifying the conception of oxidation-reduction potentials is false, for the potential does not vary with the pH of the solution as it would were the electrode acting as if it were a hydrogen electrode. On the other hand, if we consider the potential of an electrode immersed in a mixture of indigo and indigo white as if it were comparable with an electrode in a ferrous-ferric iron solution, we get no constant if we pay no attention to the pH of the solution (Clark 1920). In this instance the electrode conducts itself as if it were a hydrogen electrode under greatly diminished hydrogen pressure, and the potential changes with change in the pH of the solution. The effect cannot be accounted for wholly on the basis of a change in the ratio of the indigo products. To extrapolate into this region relations such as are found in the ferrous-ferric iron mixture would be as false as it is to extrapolate the relations of a hydrogen electrode into the ferrous-ferric iron region of reduction potential.

On the other hand the pH of a medium may alter the concentrations of ions effective in establishing an oxidation-reduction equilibrium, as Stieglitz (1917), has indicated is the case in the activation of the reducing power of formaldehyde.

Thus the pH of a solution is of importance to oxidation-reduction potentials from two points of view.

In passing, it may be mentioned that the instruments and many of the principles which are being here described for the determination of hydrogen ion concentration are applicable in the determination of oxidation-reduction equilibria and in the titration of oxidizing or reducing substances. The oxidation-reduction electrode with potentiometric measurement has been applied extensively to the determination of the end points of titrations and to the study of oxidation-reduction equilibria. Some recent work showing the effect of pH is that of Clark (1920).

From what has been said in regard to the relation of the hydrogen electrode to oxidation-reduction electrodes it is evident that a very thorough reduction of a solution must take place before true hydrogen electrode potentials at equilibrium can be established. It is fortunate, however, that the hydrogen electrode, if furnished an abundance of hydrogen and allowed to come in contact with every part of the solution, reduces rapidly especially if the principle of Eggert as applied in Clark's vessel be applied. If it does

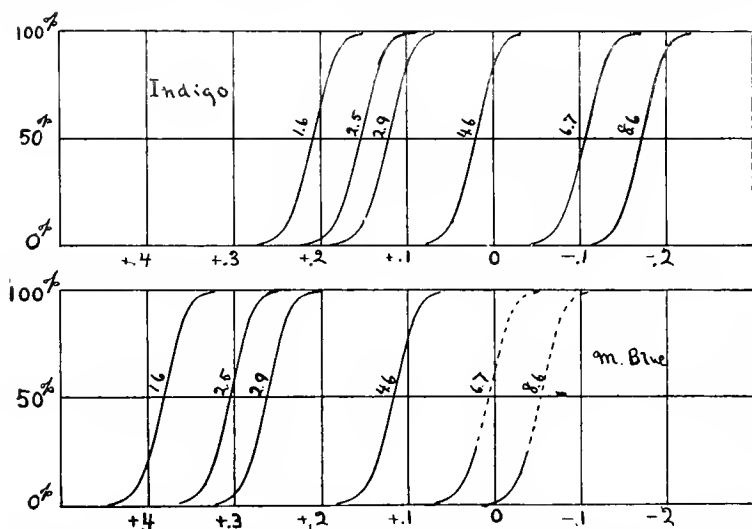


FIG. 35. RELATION OF pH TO OXIDATION-REDUCTION EQUILIBRIA OF INDIGO-INDIGO WHITE AND METHYLENE BLUE-METHYLENE WHITE

Abcissas: reduction potential. Ordinates: percentage reduction. Figures on curves: pH values.

not reduce fully, it does, in most biological solutions, reduce to an extent which permits the establishment of a virtual hydrogen electrode equilibrium from the potential of which the pH of a solution can be calculated. The author's study of bacteriological culture media seems to fully justify this conclusion. Nevertheless there are special fluids which require the greatest caution. The interfering action of haemoglobin has been recognized by students of the pH of the blood. The interfering action of other oxidizing material in other cases has not always been given the attention it deserves.

CHAPTER XV

SOURCES OF ERROR IN ELECTROMETRIC MEASUREMENTS OF pH

Besides faults in the potentiometric system there are a variety of sources of error which demand special attention. Some of these are specific to hydrogen electrode work; others are not.

Sometimes the most trivial occurrence may cause considerable trouble; such is the bubble of gas that may persistently cling to the bore of a stopcock key which is part of a liquid connection. This is mentioned simply to emphasize the constant watchfulness required of the operator of a hydrogen electrode system. A well-shielded electrical system may be put out of commission in the most unexpected way. Miserly supply of hydrogen with which to sweep out hydrogen electrode vessels is perhaps one of the commonest faults, but the hoarding of solutions which should be used to rinse away the buffer action of solutions previously used in a vessel may also be serious.

Aside from such questions of technique there are certain inherent difficulties in the application of the hydrogen electrode method. There is hardly any use attempting the measurement of unbuffered solutions, if indeed there would be any significance to the measurement were it accurate. There are other solutions which are so obviously unsuited for measurement that no attempt would be made. These are solutions containing known, strong oxidizing agents. Such solutions however grade into those with not very apparent oxidizing properties and in dealing with these one must be on guard.

The hydrogen electrode if properly treated gives such a precisely defined potential in certain well buffered inorganic solutions, reaches this potential so rapidly, returns when polarized, and adjusts itself to temperature and pressure changes so well that there is little doubt of its being a reversible, accommodating, relatively quick acting electrode. It is perhaps because of this that it shows a hydrogen electrode potential in solutions which could be slowly reduced by hydrogen. For instance certain culture media may exhibit upon an electrode of platinum uncharged with hydrogen

a potential which is distinctly toward the oxidizing region of oxidation-reduction potential. That they are capable of reduction and that the first potential is not a pseudo potential is shown by the orderly progress of the potential toward that of a hydrogen electrode under the activity of bacteria. Yet such culture media if treated in the first place as in making a hydrogen electrode measurement exhibit a fairly constant and reproducible potential the calculated pH value from which checks well with colorimetric measurements. The explanation seems to be that although that complete reduction of material to a point where the oxidation-reduction equilibrium will support an atmosphere of hydrogen is not attained, there is established a virtual hydrogen electrode equilibrium by reason of the rapidity of action between hydrogen and hydrogen ion and the slowness of action between hydrogen and oxidizing agents.

The effect of an intense oxidizing agent will be at once recognized. At the other extreme are the cases where no drift in the E. M. F. in the direction of an oxidizing action at the hydrogen electrode will be detected. Between these extremes lie the subtle uncertainties which make it advisable to check electrometric measurements with indicator measurements and to apply tests of reproducibility, of the effect of polarization, of the effect of time on drift of potential and all other means available to establish the reliability of an electrometric measurement in every doubtful case.

There are effects of unknown cause which are included under the term "poisoned electrodes." An electrode may be "poisoned" by a well defined cause such as those to be mentioned presently; but occasionally an electrode will begin to fail for reasons which cannot be traced. There is hardly any way of putting an observer on his guard against this except to call his attention to the fact that if he is familiar with his galvanometer he will notice a peculiar drift when balancing E. M. F.s.

Arsenic deposits, adsorption of material by the platinum black (with such avidity sometimes that redeposition of the black is necessary), the deposit of films of protein; have all been detected as definite causes of electrode "poisoning." Michaelis (1914) places free ammonia and hydrogen sulfid among the poisons. They undoubtedly are, but there may be involved a distinction between a poison in the sense used up to this point and a poison in

the sense that the material in question acts electromotively. Oxygen acts thus and no true hydrogen electrode can be established in its presence. Likewise other gases may act electromotively and then must be displaced before a true hydrogen electrode equilibrium can be obtained. The catalytic action of the electrode and the abundant supply of hydrogen can be depended upon only to deal with *traces* of oxygen and other gases. Gases such as nitrogen and carbon dioxide are generally treated as dilutents of the hydrogen atmosphere and in very precise measurements must be taken into account if not completely displaced. Carbon dioxide of course also has an effect upon the hydrogen ion equilibria of the solution.

Of the antiseptics used in biological solutions Michaelis (1914) states that neither chloroform nor toluol interfere if dissolved. He does not mention that chloroform may hydrolyze to hydrochloric acid. Drops of toluol however affect the electrode. Phenol is permissible but of course in alkaline solutions participates in the acid base equilibria.

The criterions of a good hydrogen electrode measurement are difficult to place upon a rigid basis but certain practical tests are easy to apply. Reproducibility of an E. M. F. with different electrodes and different vessels is the foremost test of reliability, but not a final test. Second is the stability of this E. M. F. when attained. It is not always practicable to distinguish between a drift due to alteration in the difference of potential at liquid junctions and a drift at the electrode but in most cases the drift at the liquid junction is less rapid and less extensive than a drift at the electrode when the latter is due to a failure to establish a true hydrogen-hydrogen ion equilibrium. A test which is sometimes applied is to polarize the hydrogen electrode slightly and then see if the original E. M. F. is reestablished. This may be done sufficiently well by displacing the E. M. F. balance in the potentiometer system. Where salt and protein errors do not interfere the gross reliability of a hydrogen electrode measurement may be tested colorimetrically. This checking of one system with the other is of inestimable value in some instances as it has proved to be in the study of soil extracts. There the possibilities of various factors interfering with any accurate measurement of hydrogen ion concentration dimmed the courage of investigators until Gil-

lespie (1916) demonstrated substantial agreement between the two methods. Subsequent correlation of various phenomena with soil acidity so determined have now established the usefulness of the methods.

In addition to the tests so far mentioned there remains the test of orderly series. Certain of the general relations of electrolytes are so well established that, if a solution be titrated with acid or alkali and the resulting pH values measured, it will be known from the position and the shape of the "titration curve" whether the pH measurements are reasonable or not. This of course is a poor satisfaction if there is any reason to doubt the measurements in the first place but it is a procedure not be scorned.

In dealing with protein solutions Robertson (1910) found that the electrode was injured by deposits of protein which he ascribed to acid coagulation of the protein by the hydrogen ions absorbed in the platinum black from previous measurements. Robertson therefore recommends that in a series of measurements with protein solutions the series be treated from the alkaline to the acid solutions. If his explanation be true there are instances where the reverse procedure should be followed. See sections on isoelectric points.

In very many instances biological fluids contain carbonate and the double effect of the carbon dioxide upon the partial pressure of the hydrogen and upon the hydrogen ion equilibria render accurate measurements difficult unless both effects are taken into consideration and put under control.

At high acidities in the neighborhood of pH 5 carbon dioxide will have relatively little effect upon a solution buffered by other than carbonates. As the pH of solutions increase the participation of CO_2 in the acid base equilibria becomes of more and more importance. The relation of the CO_2 partial pressure in equilibrium with the carbonates of a solution is a function of both the pH and the total carbonate. If, however, we consider for the sake of the argument that the total carbonate remains fairly low and constant, the CO_2 partial pressure becomes less with increase in pH while its effect upon the hydrogen ion equilibria increases with increase in pH. Therefore it may be said that it is of more importance under ordinary conditions to maintain the original CO_2 content of the solution than it is to be concerned about the effect of CO_2 upon the partial pressure of the hydrogen. Further-

more the effect of diminishing the partial pressure of the hydrogen is of *relatively* small importance.

For these reasons the bubbling of hydrogen through the solution is to be avoided unless one cares to determine the partial pressure of CO_2 which must be introduced into the hydrogen to maintain the carbonate equilibria and then provides the proper mixture. The method usually employed is to use a vessel such as that of Hasselbalch, of McClendon or of Clark in which a preliminary sample of the solution can be shaken to provide the solution's own partial pressure of CO_2 , and in which there is provision for the introduction of a fresh sample with its full CO_2 pressure. The hydrogen supply is then kept at atmospheric pressure and the partial pressure of hydrogen in the electrode vessel is either considered to be unaffected by the CO_2 pressure or corrected from the known CO_2 pressure of the solution under examination.

Of course in cases where the total carbonate in solution rises to considerable concentrations the partial CO_2 pressure may become of very significant magnitude and its effect in lowering the hydrogen pressure must be carefully considered.

In determining the hydrogen ion concentration of the blood by the electrometric method the two outstanding difficulties encountered are the presence of carbonate and oxyhaemoglobin. If hydrogen is swept through the fluid it will remove so much of the CO_2 that the hydrogen ion concentration is lowered. If hydrogen is not swept through, the CO_2 will escape into the hydrogen atmosphere about the electrode and reduce the partial pressure of the hydrogen. The oxygen present in the oxyhaemoglobin "depolarizes" the hydrogen electrode and makes necessary a very complete reduction of the blood before a hydrogen electrode equilibrium can be attained.

To take care of the CO_2 effect Höber used hydrogen containing CO_2 at the partial pressure of the blood. Hasselbalch provided this partial pressure by shaking a portion of the blood to be examined with the hydrogen that was to be used in the measurement. To take care of the depolarization effect of the oxyhaemoglobin Michaelis recommends minimal contact of electrode and blood so that there shall be intense *local* reduction of the blood where the electrode can be provided with an abundance of hydrogen.

In some solutions the process of reduction may alter the hydrogen ion equilibria.

CHAPTER XVI

STANDARD SOLUTIONS FOR CHECKING HYDROGEN ELECTRODE MEASUREMENTS

In the routine measurement of hydrogen ion concentrations it is desirable to frequently check the system. To do so in detail is a matter of considerable trouble; but if a measurement be taken upon some solution of well defined pH, and it is found that the potential of the chain agrees with that determined by careful and detailed measurements upon all parts, it is reasonably certain that the several sources of E. M. F. are correct.

Any one of the buffer mixtures whose pH value has been established may be used for this purpose, but there are sometimes good reasons for making a particular choice.

Sørensen (1909) used a mixture of 8 volumes of standard glycol solution to 2 volumes of standard hydrochloric acid solution for the details in the preparation of which see page 78. Michaelis (1914) recommends what has come to be known as "standard acetate." This is a solution tenth molecular with respect to both sodium acetate and acetic acid. Its preparation and hydrogen electrode potential at 18°C. have been carefully studied by Walpole (1914). Walpole proposes two methods for its preparation:

(1) From N-sodium hydroxid solution free from carbon dioxide and N-acetic acid adjusted by suitable titration (using phenolphthalein), so as to be exactly equivalent to it.

(2) From N-sodium acetate and N-acetic acid adjusted by titration of a baryta solution, the strength of which is known exactly in terms of the N-hydrochloric acid solution used to standardize electrometrically the normal solution of sodium acetate.

Walpole defines N-sodium acetate as a "solution of pure sodium acetate of such concentration that when 20 cc. are taken, mixed with 20 cc. of N-hydrochloric acid, and diluted to 100 cc. the potential of a hydrogen electrode in equilibrium with it is the same as that of a hydrogen electrode in equilibrium with a solution 0.2 normal with respect to both acetic acid and sodium chloride." By mixing the N-acetate with the N-HCl in accordance with this definition and then determining the potential of a hydrogen electrode in equilibrium with it Walpole shows that the N-sodium

acetate solution may be accurately standardized. In the following table are given Walpole's values showing the relation of the E. M. F. of the chain: $\text{Hg}|\text{HgCl}|\text{KCl} (0.1\text{M})|\text{KCl} (\text{sat.})|\text{Acetate}$ H_2Pt at 18° , to the cubic centimeters of N-HCl added to 20 cc. N-sodium acetate and diluted to 100 cc. If, for instance, the potential found is 0.4800 volts, the ratio $\frac{\text{Concentration of HCl}}{\text{Concentration of Na Ac}}$

is $\frac{20.2}{20.0}$. Hence the sodium acetate is 0.9901N.

CUBIC CENTIMETERS OF N/1 HCl TO 20 CUBIC CENTIMETERS N/1 NaAc DILUTED TO 100 CUBIC CENTIMETERS	E. M. F.
19.00	0.5270
19.40	0.5155
19.50	0.5125
19.90	0.4945
20.00	0.4898
20.39	0.4712
20.89	0.4549
21.00	0.4525

These values are more convenient to use if plotted as Walpole has done.

Walpole found that the E. M. F. of the chain: $\text{Pt}|\text{H}_2$ "standard acetate" $|\text{sat. KCl}| 0.1\text{N KCl}|\text{HgCl}|\text{Hg}$ at 18°C. is 0.6046. The contact potential still to be eliminated was estimated by the Bjerrum extrapolation to be 0.0001 volt. Hence the true potential is 0.6045. This value seems to be the value of the chain corrected to one atmosphere hydrogen plus vapor pressure.

Michaelis (1914) gives the following values for the difference of potential between the saturated KCl calomel electrode and the hydrogen electrode in his standard acetate.

TEMPERATURE	E. M. F.	TEMPERATURE	E. M. F.
15	0.5170	21	0.5180
16	0.5171	22	0.5183
17	0.5172	23	0.5186
18	0.5174	24	0.5190
19	0.5175	25	0.5195
20	0.5178	34-38	0.5200-0.5205

It will be noted that both Sørensen's standard glycocoll and the standard acetate solutions must be constructed by adjustment of the components. While there is no great difficulty in this there remain the labor and the chance of error that are involved. Clark and Lubs (1916) have shown that acid potassium phthalate possesses a unique combination of qualities desirable for the standard under discussion. The first and second dissociation constants

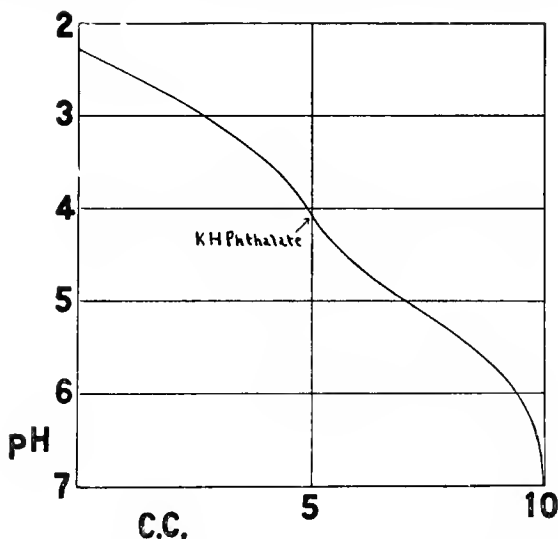


FIG. 36. TITRATION OF PHTHALIC ACID WITH KOH

are so close to one another that the second hydrogen comes into play before the first is completely neutralized (see fig. 36). As a consequence the half neutralized phthalic acid (KHPthalate) exhibits a good buffer action. The salt of this composition crystallizes beautifully without water of crystallization, and, as was shown by Dodge (1915) and confirmed by Hendrixson (1915) it is an excellent substance for the standardization of alkali solutions. As such it is used to standardize the alkali entering into the buffer mixtures of Clark and Lubs (see page 72). The outstanding feature is that the ratio of acid to base is fixed by the composition of the crystals and not by adjustment as in other standards. The salt may be dried at 105°C. and accurate concentrations constructed. The diffusion potential against satu-

rated KCl is somewhat higher than that of standard acetate as estimated by the Bjerrum extrapolation but not so high as to make good readings difficult.

Clark and Lubs (1916) found for the chain:

$\text{Hg}|\text{HgCl}|\text{KCl (saturated)}||\text{M}/20\text{ KHPhtalate}|\text{H}_2|\text{Pt}$
at 20°C. and E. M. F. of 0.4807 corrected to one atmosphere of hydrogen. Their saturated calomel electrode was 0.0882 volt more negative than the average of a set of tenth normal calomel electrodes. Assuming 0.3379 (cf. Chapter XVII) as the value of the tenth normal calomel electrode and 0.0004 volt for the diffusion potential still to be eliminated, the hydrogen electrode potential of M/20 KHPhtalate at 20° is 0.2306.

Unfortunately the temperature relations of such chains are not accurately known. For ordinary work the pH of M/20 KHPhtalate may be considered as 3.97 between 20° and 30°C. Assuming a liquid junction potential difference of 0.0004 volts we can reckon from this the following total electromotive forces at various temperatures of the chain:

Calomel electrode of KCl conc. X	Sat. KCl	Hydrogen electrode at one atmosphere in KHPhtalate ($\frac{m}{20}$)
----------------------------------	----------	--

TABLE 14

TEMPERATURE	TOTAL E. M. F.		
	X 0.1M	X 1.0M	X saturated KCl (approximate)
18	0.5675	0.5158	0.4800
20	0.5689	0.5170	0.4802
22	0.5704	0.5181	0.4806
24	0.5719	0.5192	0.4812
26	0.5733	0.5204	0.4817
28	0.5748	0.5215	0.4822
30	0.5763	0.5227	0.4827

These values are entirely provisional for temperatures other than 20°C. and require experimental verification before they can be used for precise standards. They are given as convenient standards for ordinary check measurements.

CHAPTER XVII

STANDARDIZATION OF pH MEASUREMENTS

In the development of the theory of electrolytic dissociation the hydrogen electrode came upon the scene comparatively late and after many of the quantitative relations had been established by conductance data. It was therefore natural that these data should have been accepted in the standardization of potentiometric measurements. It now appears that the interpretation of conductance data is more complicated than at first supposed and that certain of the values that have been used in the standardization of potentiometric measurements are in doubt. The resulting confusion demands careful consideration.

Let us review briefly the way in which conductance data enter into the potentiometric system.

We have no convenient and reliable means of determining the absolute difference of potential between a hydrogen electrode and the solution in which it is immersed. We are therefore forced to set up a concentration chain and to measure the algebraic sum of the two potential differences at the two electrodes. By the relation

$$E = \frac{RT}{nF} \ln \frac{C_1}{C_2}$$

we obtain the *ratio* of two hydrogen ion concentrations if the solutions are sufficiently dilute to permit the application of the gas laws from which the above equation was derived. To apply this equation directly to the determination of either concentration C_1 or C_2 the other concentration must be known. Conductance data have been relied upon to furnish the known concentration.

Likewise, when a chain composed of a calomel electrode and a hydrogen electrode is used, the value assigned to the calomel electrode is such that when it is subtracted from the total E. M. F. of the chain the resulting E. M. F. is as if between a normal hydrogen electrode and the hydrogen electrode under measurement. The equation of such a concentration chain is

$$E = \frac{RT}{nF} \ln \frac{1}{C_x}$$

This implies the experimental determination of the difference of potential between a normal hydrogen electrode and the calomel electrode or else between the calomel electrode and a hydrogen electrode in some solution of *known* hydrogen ion concentration. To determine this known hydrogen ion concentration conductance data upon hydrochloric acid solutions have been relied upon.

Unfortunately hydrochloric acid solutions exhibit the so-called anomalies of strong electrolytes which have already been mentioned. Although it was known from the first that hydrochloric acid solutions do not obey the dilution law, it was supposed that the ratio of the equivalent conductances at volume v and at infinite dilution (where there is complete dissociation) would give the percentage ionization at volume v and hence the hydrogen ion concentration at this dilution v . However, this conclusion involves the assumption that the mobilities of the ions remain unaltered between dilution v and infinite dilution. Jahn (1900) and Lewis (1912) have questioned this assumption and within recent years the conclusion has become firmly established among many investigators that the mobilities do change or else that the chemical activity of the ions of strong electrolytes is not strictly proportional to their concentration. In other words conductance data alone are not sufficient to define with precision the hydrogen ion concentrations of the hydrochloric acid solutions which have been used to standardize the hydrogen electrode system of concentration chains. In support of this contention there have been brought forward comparisons of the concentration chains themselves. There is evidence that the ratio $\frac{C_1}{C_2}$ in the concentration chain formula,

$$E = \frac{RT}{nF} \ln \frac{C_1}{C_2}$$

is not necessarily determined with accuracy when a measurement of the E. M. F. of such a chain is taken. What is it then that is determined? The way in which this question will be answered will doubtless form another interesting chapter in the philosophy of science. Focused upon this point are two tendencies; the one

seeking to find the factors which interfere with the application of the simple gas laws so that the experimental data may be corrected to apply to the ideal; the other seeking to formulate either the empirical data or the thermodynamic relations without special reference to the mechanisms involved.

It was an astute suggestion of Lewis (1907) that the simple thermodynamic relations be assumed to hold, not for concentration pressure relations, but for quantities which, when introduced into the equations embodying the gas laws, will make these laws apply. The two new quantities are *activity* and *fugacity*. In the special case of a "perfect" solution, a very dilute solution, obeying the laws of gases, activity and fugacity are equal to concentration and pressure respectively. But when a solute ceases to conduct itself in accord with the laws of gases, its fugacity and activity remain such that the equations which apply to "perfect" solutions still hold.

Stated in the above manner it may appear to those who insist upon looking for the means of applying concentration relations as if Lewis had made use of a clever dodge. In reality he has simply expressed in a form which he has developed into a self-consistent system that which is the more directly determined experimentally. This is at once evident in the definition of activity by the following postulates.

1. When the activity of a substance is the same in two phases, that substance will not of itself pass from one phase to the other. 2. When the activity of a substance is greater in one phase than in another, the substance will pass from the one phase into the other, when they are brought together.

With these postulates Lewis proceeds to develop a self-consistent system in which it appears that in a "concentration cell" the ratio of activities is related to the E. M. F. by the equation

$$\text{E. M. F.} = \frac{RT}{nF} \ln \frac{\text{activity 1}}{\text{activity 2}}$$

Only at infinite, or very high dilution, when a solution approaches an "ideal" solution, does the more familiar relation of concentration hold true in the equation

$$E. M. F. = \frac{RT}{nF} \ln \frac{C_1}{C_2}$$

So long as the limitations were well understood it was permissible to speak of the hydrogen electrode method as a means of determining relative concentrations. If one is willing to use Lewis' terms he would be more precise to speak of the hydrogen electrode method as a means of determining relative hydrogen ion activities. If, then, we refer electrode potentials to the normal hydrogen electrode it is necessary for precision that we redefine normal (molar) hydrogen ion concentration as Lewis and Randall have done in the following manner: "A solution is said to be at (hypothetical) molar concentration with respect to hydrogen ion when the activity of hydrogen ion in this solution is n times as great as in $1/n$ M solution of hydrogen ion, where n is a large number." Furthermore in using some well defined hydrochloric acid solution for the hydrogen electrode standardization of a calomel electrode it is necessary to find the corrected degree of dissociation and to abandon the assumption that the conductance data formerly used can be applied in the simple manner hitherto practiced.

Using the most probable values for the corrected degree of dissociation of hydrochloric acid solutions, the E. M. F. of the cell: normal calomel electrode-hydrogen electrode in N/10 or N/100 HCl, and the estimated contact potential difference at the liquid juncture, Lewis and Randall obtained the value 0.2776 for the difference of potential between the normal calomel and the normal hydrogen electrodes at 25°. This value was revised to 0.2828 by Lewis, Brighton and Sebastian (1917). Direct comparison with N/10 KCl calomel electrode as will be noted later gave 0.3357 as the potential value of this electrode including a slight liquid junction potential difference.

Now let us consider the values hitherto used in biochemical work.

In Sørensen's work, published prior to the adoption of the present standard value of the Weston standard cell, the basis for the particular cell whose value he gave was not stated. If it was the 1.01863 used in Germany prior to 1911 the correction of Sørensen's data to the present international volt will not be significant. Doubtless the international standard was used in Denmark when

Sørensen (1912) published the summary of the data of Sørensen and Koefoed. Their values involve two assumptions; first that liquid junction potential differences were eliminated by the Bjerrum extrapolation; second, that in the calculation of the theoretical difference of potential between the normal hydrogen electrode and the hydrogen electrode in the hydrochloric acid solutions used, the correct hydrogen ion concentration was given by conductance data. As already stated there is serious doubt of the validity of the last assumption. Even so we ought, by using the same degree of dissociation for hydrochloric acid solutions, to reconcile Sørensen's value with that of Lewis, Brighton and Sebastian. Sørensen assumed 91.7 per cent dissociation of 0.1M HCl at 18°C. Employing the same value at 25°, as an approximation, we would find that the hydrogen electrode in 0.1M HCl should be 0.0614 volts more negative than a "normal" hydrogen electrode. If however we take "the corrected concentration of H^+ in 0.1M HCl as 0.0816" (Lewis, Brighton and Sebastian) then the difference would be 0.0643. The correction 0.0029 should bring Sørensen's value into harmony with that of Lewis, Brighton and Sebastian. However, they are:

Lewis, Brighton and Sebastian.....	0.3357
Sørensen (corr.).....	0.3347

The discrepancy of 0.0010 volt remains to be explained. That it may be ascribed partly to an involved potential difference between N/10 KCl and N/1 KCl which has not been noted in the discussion and partly to an excess correction for diffusion potential through the use of the Bjerrum extrapolation seems probable from the treatment accorded this subject recently by Fales and Vosburgh; but if we attempt to correct Sørensen's data by the use of the curves given by Fales and Vosburgh the discrepancy noted above widens. It is of no particular importance to attempt further to reconcile the two values because Sørensen's original data (1909) show wide variations in the E. M. F.s. of the chains in which hydrochloric acid was used. One might therefore jump to the conclusion that Sørensen's value is unworthy of further consideration now that we have a more probable value. It must be emphasized however that we are not so much concerned with the reliability of Sørensen's original data as we are with the fact that

the value thereby assigned to the tenth normal calomel electrode has been widely used in the study of hydrogen electrodes in solutions which exhibit comparatively low diffusion potentials against KCl and which furnish hydrogen electrode potentials reproducible with a considerable degree of precision. Because of this, because of the fact that the Sørensen value and other comparable values have standardized an enormous amount of biochemical data we regard it as important to consider the old value further.

When Sørensen's value has not been used directly it has been used indirectly in the taking over of pH values assigned to standard solutions such as standard acetate. In Walpole's study of acetate mixtures he appears to have been consistent in using the value assigned by Sørensen to the tenth normal calomel electrode referred to the normal hydrogen electrode under one atmosphere of hydrogen plus vapor pressure. He obtained a value for the hydrogen electrode potential in standard acetate agreeing with that found by Sørensen and by Michaelis. In Clark and Lubs' study of phthalate, phosphate and borate buffer mixtures they applied the Bjerrum extrapolation, and, with the qualifications stated in their paper reached a value¹ for their tenth normal calomel electrode in substantial agreement with Sørensen.

Palitzsch doubtless used the Sørensen value, which he originally aided in determining, in his study of borate buffer mixtures.

A variety of similar channels might be followed to show that in the biochemical literature there is substantial agreement so far as the assumed difference between the tenth normal calomel and the normal hydrogen electrodes is concerned. Since the liquid junction potential differences between saturated KCl and the buffer solutions and physiological fluids dealt with in biochemistry are of a low order of magnitude it seems fair to assume that the more precise biochemical data are fairly well *standardized*, though not necessarily accurate. The agreement was furthermore encouraged by the recommendation of Auerbach (1912) when, in his summary of the work of the "Potential Commission,"

¹ Clark and Lubs give their E. M. F. s reduced to refer to the normal hydrogen electrode under a standard hydrogen *concentration* rather than the standard pressure usually used. Since the calomel values were also referred to the same basis the pH values given by these authors remain as if the customary procedure had been followed.

he recommended the use of the tenth normal calomel as a working standard because of its low temperature coefficient, and assigned the value 0.337 for use between 20° and 30°

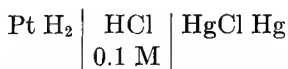
On the one hand, then, we have what may be regarded as a tacitly accepted and not yet precisely formulated standardization of the tenth normal calomel electrode; and on the other hand a distinctly different value for the tenth normal calomel electrode that is doubtless more nearly correct, though the details by which the value was reached are not presented. The biochemist is thus placed in an embarrassing position. Before making a choice he may consider the present situation in our knowledge of the temperature coefficients of calomel electrodes.

In dealing with the temperature coefficients it will be distinctly understood that we are not concerned with the temperature coefficient of the absolute difference of potential between mercury and solution but rather with the temperature coefficient of the calomel electrode in the cell: calomel electrode-normal hydrogen electrode, when the potential difference at the normal hydrogen electrode is *defined* to be zero *at all temperatures*. Unfortunately we have little data upon this temperature coefficient which is both accurate and extensive. Therefore one who chooses to take over the better value for the tenth normal or the normal calomel electrode will still be left in the predicament of not knowing the precise value to use at temperatures other than 25°C.

We can only reach approximate values in the following manner and compare the results with comparatively old experimental data.

Lewis and Randall (1914) have derived a provisional temperature coefficient for the normal calomel electrode which indicates that the values are not a linear function of the temperature. The derivation of these authors as applied to the tenth normal electrode will be followed but some new values obtained since the writing of their paper will be introduced.

For the cell



Lewis and Randall give the empirical equation

$$E = 0.0964 + 0.001881T - 0.000,00290T^2$$

whence

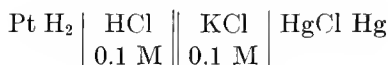
$$dE/dT = 0.001881 - 0.00000580T$$

For present purposes this conforms closely enough with Ellis' (1916) data.

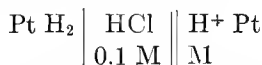
It is now assumed that the temperature coefficient of the cell



will apply to



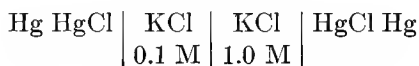
if the tenth molar hydrochloric acid calomel cell has the same potential as the tenth molar KCl calomel cell. Compare however Lewis, Brighton and Sebastian (1917) who give 0.0012, and MacInnes (1919) who gives 0.0. For the cell



Lewis, Brighton and Sebastian give 0.0644. Assuming that in this cell the E. M. F. is proportional to the absolute temperature $\frac{dE}{dT} = 0.00022$. Hence for the tenth molar KCl calomel electrode against the normal hydrogen electrode

$$\frac{dE}{dT} 0.00166 - 0.00000580T.$$

For the cell



the author finds at 20° 0.0519, and at 30° 0.0536. Interpolation between these values on the assumption that the E. M. F. is a linear function of the temperature gives an E. M. F. at 25° which is within 0.15 millivolts of that found by Lewis, Brighton and Sebastian, and a linear temperature coefficient of 0.000,17. Sauer's value at 18° is 0.0514 and that of Fales and Vosburgh at 25° is 0.0524. Neither of these values fall in with these mentioned above but when taken by themselves and with the 15° value,

0.0509, given in the footnote of the paper by Fales and Vosburgh (1918) they furnish a temperature coefficient of the same order.

With these data we can start from the value 0.2828 as that of the normal calomel electrode (Lewis, Brighton and Sebastian, 1917) at 25°; or with Sørensen's (1912) value, 0.3380, for the tenth normal calomel electrode at 18° and treating each set separately we reach the following comparisons:

TABLE 15

LEWIS			TENTH AGAINST NORMAL CALOMEL	SÖRENSEN		
t	1.0 N	0.1 N		1.0 N	0.1 N	0.1 N Found
18	0.2844	←0.3360	0.0516	0.2864	←0.3380	0.3380
		↑			↓	
20	0.2840	←0.3359	0.0519	0.2860	←0.3379	0.3378
		↑			↓	
25	0.2828	→0.3356	0.0528	0.2848	←0.3376	
		↓			↓	
30	0.2817	←0.3353	0.0536	0.2837	←0.3373	0.3370
					↓	
40					0.3360	0.3359
					↓	
50					0.3341	0.3344
					↓	
60					0.3317	0.3321

Approximate temperature coefficient of normal calomel electrode -0.000,23.

Approximate temperature coefficient of tenth normal calomel electrode -0.000,06.

Bjerrum's values at 0°, 25° and 75° do not fit in with the calculations given above.

The values given above are admittedly uncertain and are to be regarded as provisional in lieu of the experimental data that is needed. It may be emphasized however that there is good reason to believe that the temperature coefficient for the tenth normal electrode is much lower than that of the normal calomel electrode.

Since we can as yet only make a good guess of the temperature relations it seems wise to choose as a standard the calomel electrode with the smaller temperature coefficient and thus lower the

chances of error. This fortunately has been, for the most part, the practice in biochemical work although it runs counter to preferences which will not be discussed.

Let us then assume that this half cell, the tenth normal calomel electrode, is to be the standard to which all working electrodes are to be referred and let us consider finally the choice of values to be assigned.

At 25°C. the difference between the values for the tenth normal calomel electrode given in table 15 is 2 millivolts. A change of this amount would shift the values in the pH scale 0.03 unit pH. This is quite insignificant or within the experimental error in many biochemical studies. For certain purposes it is not insignificant. When carried into mass action relations it might be serious *but* in such relations there are generally involved data taken over from conductance measurements. In such a situation therefore there are involved complexities which are by no means covered by the mere selection of a more probable value for the standard electrode.

We have already mentioned the fact that even if the value of Lewis, Brighton and Sebastian be absolutely correct at 25° we cannot assign accurately known values at temperatures other than 25°, and we have noted the more or less tacit assumption of standard values for various temperatures in the course of the development of biochemical applications.

It is our conclusion that in the absence of sufficient data to formulate a comprehensive set of true values, and in the absence of concerted action in regard to the calomel electrode such as that which fixed the present *standard* value of the Weston cell, it will be advisable to fall in with a *standard* which has been tacitly accepted. The author therefore suggests that the values in column 6 of table 15 be used as provisional standards wherever there is no definite reason to *require* any other value.

We can thus preserve uniformity in pH data and not introduce ill considered changes which may need subsequent frequent revision before the present theoretical difficulties are removed or before the action of an international committee fixes a standard value.

It may be objected that under such a procedure of standardization the symbol pH loses the precise significance which has been

attached to it. It has always been defined as $\log \frac{1}{[\text{H}^+]}$. If the "concentration chain" does not determine with precision the ratio of two hydrogen ion concentrations but rather the ratio of two hydrogen ion activities, and if, in addition, we adopt a standard of reference in the current use of the hydrogen electrode which is not strictly true, then pH is no longer expressive of the true value of $\log \frac{1}{[\text{H}^+]}$. We need not be concerned with the casuistry of this situation. We need only remember that the more precise uses to which hydrogen electrode measurements may be put involve theoretical difficulties which we are not yet prepared in every case to deal with accurately, that in the more common uses the uncertainty is not of a serious magnitude and that it is preferable to maintain uniformity in the manner of stating *experimental values*. If we take care to put a definite and unequivocal meaning to experimental data, relieving them as far as possible from ill-defined presumptions, we may be pardoned for continuing to use in descriptive text and in approximate calculations "hydrogen ion concentrations." When we come to exact statements they will be found embodied in pH values of uniform experimental derivation.

In summary then it is suggested that:

1. The following values shall be taken as the *standard* differences of potential, liquid junction potential differences being eliminated; between a tenth normal KCl calomel electrode and a hypothetical hydrogen electrode immersed in a solution normal with respect to the hydrogen ions, under one atmosphere partial pressure of hydrogen, and considered to have zero difference of potential between electrode and solution at all temperatures.

	TEMPERATURE				
	18°	20°	25°	30°	40°
Potential difference.....	0.3380	0.3379	0.3376	0.3373	0.3300

2. The standard experimental meaning of pH shall be the corrected difference of potential between the hypothetical normal

hydrogen electrode and the hydrogen electrode under measurement, when this difference is derived by the use of the above values divided by the numerical quantity 0.000,198,37 T.

3. In every case it shall be specified whether the Bjerrum extrapolation with the use of 1.75 and 3.5N KCl was used to eliminate liquid junction potentials or whether saturated KCl was used and considered to eliminate liquid junction potentials.

There are those who will prefer to use the saturated KC calomel electrode as a working standard. Its use eliminates the protective devices required to guard the tenth normal calomel electrode against the saturated KCl used as a liquid bridge. Michaelis (1914) has also noted that its temperature coefficient is such that it tends to balance the effect of fluctuations in the temperature of a calomel electrode-hydrogen electrode chain. Though there are involved in Michaelis' reasoning some factors which are yet uncertain this advantage may be granted. A practical system which embodies the merits of the saturated calomel electrode and which meets the requirements of the standardization suggested above is illustrated on page 129. In this system the saturated calomel electrode is the working standard whose value is given by careful comparison at known temperatures with a set of tenth normal calomel electrodes.

These suggestions simply put into definite form the current procedure with the recognition on the one hand that the precise use of electrode data involve many theoretical difficulties and on the other hand that the use of such data for the approximate calculation of hydrogen ion concentrations had best be standardized for the sake of uniformity in the records to be handed on to the future.

CHAPTER XVIII

SUPPLEMENTARY METHODS

When any process has been found to be controlled by the concentration of the hydrogen or hydroxyl ions, when the quantitative relations have been established and contributory factors are controllable, there is established a possible means of estimating the concentration of the hydroxyl or hydrogen ions. Many such instances are known. From among them a few may be chosen for their convenience. They are spoken of here as supplementary methods because they are superseded in general practice by indicators and the hydrogen electrode. Several have historical value because they were used in establishing the laws of electrolytic dissociation. Others have value because they are available either for checking the customary procedures or for determinations in cases where there is reason to doubt the reliability of indicator or hydrogen electrode measurements.

An instance of the procedure outlined above is the following. Clibbens and Francis (1912) found that the decomposition of nitrosotriacetoneamine into nitrogen and phorone is a function of the catalytic activity of hydroxyl ions. Francis and Geake (1913) then applied the relation to the determination of hydroxyl ion concentrations, Francis, Geake and Roche (1915) improved the technique, and then McBain and Bolam (1918) used the method to check their electrometric measurements of the hydrolysis of soap solutions.

It is just in such checking that the value of these so-called supplementary methods will be appreciated. But, since they will find only occasional use and under circumstances which will require a detailed consideration of their particular applicability, there seems to be no reason to do more than indicate a few of the methods in brief outline.

CONDUCTIVITY

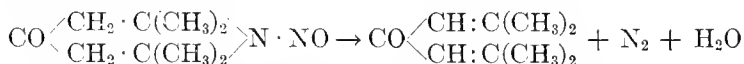
The conductivity of a solution is dependent upon the concentrations of all the ions and upon the mobilities of each. It is therefore obvious that a somewhat detailed knowledge of the con-

stituents of a solution and the properties of the constituents is necessary before conductivity measurements can reveal any accurate information of the hydrogen or hydroxyl ion concentration. Even when the constituents are known it is a matter of considerable difficulty to resolve the part played by the hydrogen ions if the solution is *complex*. However, the mobilities of the hydrogen and hydroxyl ions are so much greater than those of other ions (see page 113) that methods of approximation may be based thereon. If, for instance, a solution can be neutralized without too great a change in its composition it may happen that with the disappearance of the greater part of the hydrogen ions there will appear a great lowering in conductance. Then, with the appearance of greater hydroxyl ion concentration, the conductance will rise. The minimum or a "knickpunkt" in the curve is a rough indication of neutrality. Thus the conductivity method is sometimes useful in titrations. It has so been used, for instance, by Dubroux (1917) in the titration of wines and by others where there is reason to believe that indicator and hydrogen electrode methods are inapplicable. Compare Küster and Grütters (1903) and Kolthoff (1920).

The elementary principles of conductivity measurements will be found in any standard text of physical chemistry but the more refined theoretical and instrumental aspects are only to be found by following the more recent journal literature.

CATALYTIC DECOMPOSITION OF NITROSOTRIACETONAMINE

The reaction taking place is represented in outline by the following equation:



The original quantity of nitrosotriacetoneamine is known and the extent of the decomposition at the end of measured intervals of time is measured by the volume of nitrogen evolved.

Francis, Geake and Roche (1915) use the vessel shown in figure 37. The tap of the reaction vessel contains a cup B of 7 to 10 cc. capacity into which the alkali or the nitrosoamine can be introduced through F. The solution is then shut in by turning the key

through a right angle. The cup becomes a part of the reaction chamber A on turning the key as shown in the figure. The vessel is immersed in a thermostat and shaken during the whole experiment. The holes at E and E' permit the cup B to be bathed by the thermostat liquid and so reach thermal equilibrium at the same time as the chamber A. The tube R connects with a con-

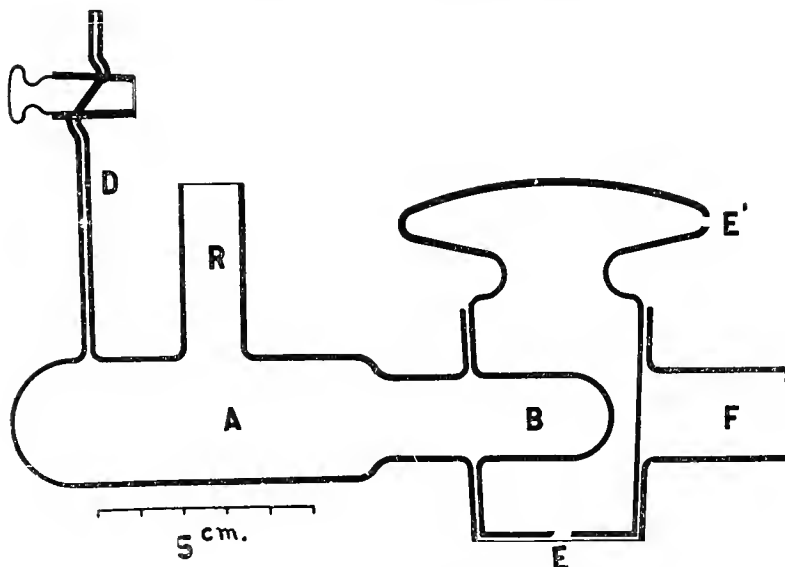


FIG. 37. VESSEL FOR THE CATALYTIC DECOMPOSITION OF NITROSOTRIACETONAMINE

stant volume burette where the evolved nitrogen is collected and its pressure read. The tube D is used for washing out the vessel and for filling it with nitrogen when the reaction has to be conducted in an atmosphere free from oxygen.

The unimolecular equation, using the pressure method is

$$k = \frac{2.303}{t} \log \frac{P_{\infty} - P_0}{P_{\infty} - P_t}$$

where P_0 is the pressure at the time taken as zero, P_t the pressure taken at the time t and P_{∞} the so-called infinity reading at the end of the experiment. The unit of time taken is a second. At

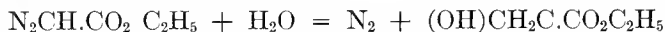
$$30^{\circ} \quad \frac{k}{[\text{OH}^-]} = 1.92.$$

It was found that the constants obtained with nitrosotriacetoneamine commence to drift when the ion concentration reaches 0.05N while at 0.35N the drift ceases and the method is again applicable. To bridge the gap it was found that nitroso-vinyl- and isobutyl-diacetonamines could be used.

For temperature coefficients and for the influence of neutral salts etc. the original paper may be consulted.

CATALYTIC DECOMPOSITION OF DIAZOACETIC ESTER

Bredig and Fraenkel (1905) have described the following reaction as applicable to the determination of hydrogen ion concentrations.



The nitrogen evolved from time to time is measured and the values used in the equation

$$k = \frac{1}{0.4343 t} \log \frac{a}{a - x}$$

where a is the total gas at the end of the reaction, x the gas after time, t minutes and k the reaction constant. At 25°C . $\frac{k}{\text{H}^+} = 32.5$.

The method was applied with only partial success by Höber (1900) to blood. Van Dam (1908) used it in the examination of rennet coagulation of milk.

THE INVERSION OF CANE SUGAR

This has been a favorite subject of study by those interested in the catalytic activity of the hydrogen ion. It has been used in a number of instances for the determination of the hydrogen ion concentration of biochemical solutions, but, like all catalytic processes, its close study has revealed a number of complicating factors which necessitate the greatest caution in the interpretation of results. According to the work of Senter, of Acree and of others the undissociated portion of an acid may play an extensive part in catalyses and the effect of neutral salts (Arrhenius 1899) is not always easy to interpret. So numerous are

the papers dealing with the theory and application of sugar hydrolysis that the reader is referred to the very thorough review by Woker.

MISCELLANEOUS METHODS

Were it worth while there could be compiled under this heading a wide variety of phenomena which have actually been used to determine approximately the hydrogen ion concentration of a solution. We may instance the precipitation of casein from milk by the acid fermentation of bacteria. This has not been clearly distinguished in all cases from coagulation produced by rennet-like enzymes; but, when it has been, the precipitation or non-precipitation of casein from milk cultures has served a useful purpose in the *rough* classification of different degrees of acid fermentation. In like manner the precipitation of uric acid or of xanthine has been used (Wood, 1903).

The alteration of the surface tension of solutions (Windish and Dietrich, 1919), the distillation of ammonia (Vely 1905), distribution ratios between different solvents, and various other methods have been used to furnish data for the estimation of hydrogen or hydroxyl ion concentrations.

CHAPTER XIX

APPLICATIONS

It is because of the great variety of applications in research, routine and industry that the theories and devices outlined in the previous chapters have been developed. The physical chemist sees in them the instruments of approximation or of precision with which there have been discovered orderly relations of inestimable service to the analyst and with which there have been established quantitative values for affinity or free energy. The biochemist might almost claim some of these methods as his own, not only because necessity has driven him to take a leading part in their development, but also because their application has become part of his daily routine in very many instances.

As mentioned in the preface the applications have become so numerous and in many cases so detailed that the time has come for a redispersion among the several sciences of the material that has from time to time been grouped about the activity of the hydrogen ion. This chapter therefore is written only as a cursory review which may be of service to the student in directing his attention into new channels, in showing the interdependence of specialized lines of research, and in searching the literature for the material which bears upon his particular problem.

In the compilation of the bibliography, of which this chapter constitutes an index, no attempt has been made to include all of the very numerous instances in which the activity of the hydrogen or the hydroxyl ions has been found to influence the course of specific chemical reactions, such as the hydrolysis of polysaccharides, special oxidations and condensations, or the nature and accuracy of the numerous color tests used for the qualitative recognition of special chemical groupings. The reader will find in Woker's extensive monograph, *Die Katalyse*, not only a very complete review of the widely scattered literature upon these aspects of hydrogen and hydroxyl ion activity but also an abundance of material which still remains to be reworked with the more modern methods. The student looking for problems could find few which would be

more profitable than the establishment of definite pH limits for some of the color reactions which are extensively used.

GENERAL REVIEWS. Excellent general reviews of biochemical applications are Sørensen's article in *Ergebnisse der Physiologie*, 1912, and Michaelis' monograph *Die Wasserstoffionenkonzentration*, 1914. Prideaux has compiled a great deal of valuable data in *The Theory and Use of Indicators*, London, 1917. In this English work will be found the more important matter which Bjerrum (1914) embodied in his monograph on the theory of titration and which Noyes had previously summarized in his paper "Quantitative application of the theory of indicators to volumetric analysis," (1910). The analyst will find a wealth of helpful suggestions in Stieglitz' *Qualitative Analysis*. A review of the indicator method which is of some general interest, although written specially for the bacteriologist, will be found in *The Journal of Bacteriology*, **2**, nos. 1, 2 and 3 (Clark and Lubs, 1917).

Those who desire to review the theory of electrolytic dissociation with special reference to its bearing on electrode measurements will find useful LeBlanc's *Text Book of Electrochemistry* (1907).

Among several papers which may be called classics in biochemistry there will be recognized the preeminence of Sørensen's *Etudes enzymatiques*, II, from the Carlsberg Laboratory in Copenhagen and *Das Gleichgewicht zwischen Basen und Säuren im tierischen Organismus* by Henderson of Harvard.

THE THEORY OF TITRATION is so closely allied with the more general applications of indicators and the hydrogen electrode that it may well be taken from the alphabetic arrangement to be followed and treated before taking up some general considerations.

The stress which has come to be laid upon that factor of "acidity" with which we have been dealing should not detract from the true importance of the estimation of total acidity or alkalinity by titration. Indeed the theories and the methods with which we have been concerned up to this point have clarified and improved methods of titration. In place of the old empiricism there has come a well ordered theory whose salient features may be simply illustrated.

In figure 38 are shown the titration curves of hydrochloric, acetic and boric acids, determined as outlined in Chapter I. The ordinates of figure 38 are pH values and the abscissas cubic centi-

meters of N/10 NaOH added to 10 cc. N/10 acid. At the side of the main part of the figure are representations of the color transformations of two indicators (see Chapter III).

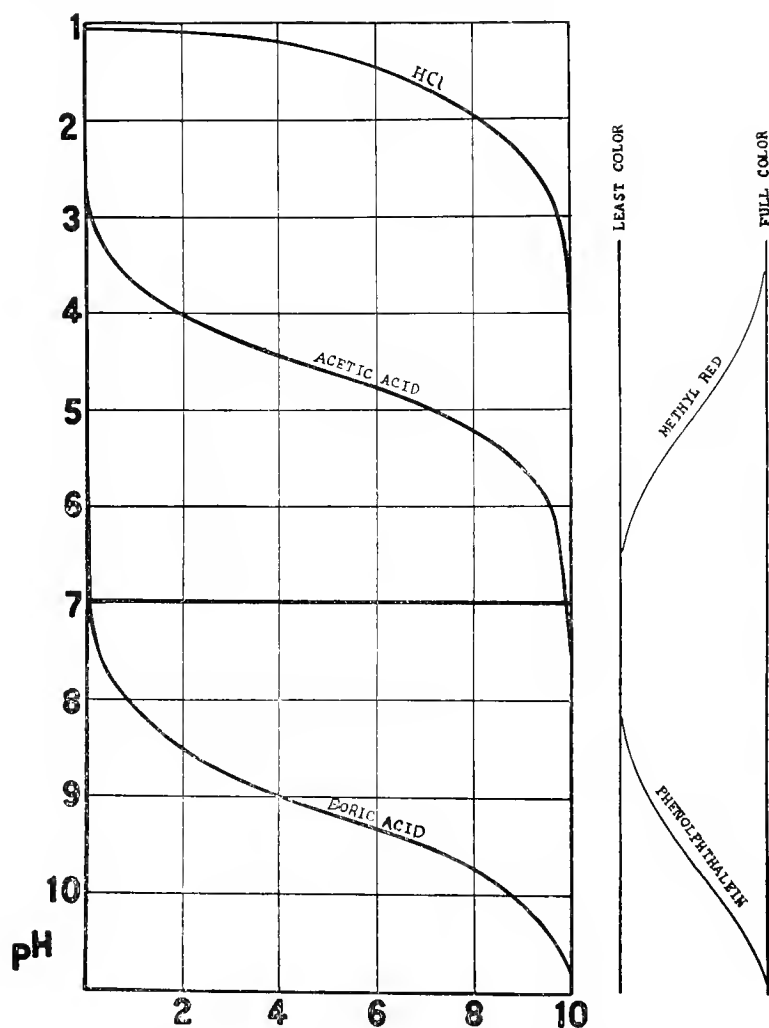


FIG. 38. TITRATION CURVES OF 10 CC. N/10 ACIDS WITH N/10 NaOH

When all but a very small part of the hydrochloric acid has been neutralized there comes a sharp break in the titration curve. On the addition of the last trace of alkali required for complete neutralization the pH of the solution plunges to the alkaline region. In this precipitous change the pH passes the range of methyl red, and, with an amount of alkali that will be detected only by careful observation, it passes into that range of pH where phenolphthalein shows its various degrees of color. Therefore, with the exclusion of carbon dioxide, either indicator may be used to indicate the "end point" of this titration. The case is very different in the titration of acetic acid. Here we have an acid whose dissociation constant (see Chapter I) is so low that the flat portion of the titration curve lies in that region of pH where methyl red shows its various degrees of color. In other words the apparent dissociation constant of methyl red is not far from that of acetic acid. Therefore, as the titration of acetic acid proceeds, and long before the neutralization of the acetic acid is complete, methyl red has been partially transformed and at last is so extensively transformed that no marked change of color is observed when the pH of the solution abruptly changes with complete neutralization of the acetic acid. It is at once evident why an indicator with the properties of phenolphthalein must be used in such a case. In the titration of a still weaker acid, such as boric acid, phenolphthalein becomes comparable to methyl red in the latter's conduct in acetate solutions. To titrate boric acid it must be combined with glycerine or mannitol to form a stronger acid. See Liempt (1920).

The titration curve of boric acid is representative of the conduct of many of the weak acidic groups found in the substances of biochemical interest. In the titration of proteins and their hydrolytic products, in the titration of culture media and the extracts of natural products the titration curve lies flat in the region of the color change of phenolphthalein and other indicators. No "end point," such as is observed in the titration of hydrochloric acid, is ever observed.

Sometimes by a judicious selection of indicators it is possible to titrate in succession a mixture of two acids. For instance A. B. Clark and Lubs (1918) have called attention to the advantages of the two color transformations of thymol blue. The color transformation of thymol blue in the acid range is such that it may be

used to indicate the approximate end point of hydrochloric acid in the presence of acetic acid; and the second color change occurs in a region of pH such that it will indicate the end point in the titration of the acetic acid. A. B. Clark and Lubs (1918) and Lubs (1920) have examined other similar uses of this indicator.

The principles thus briefly outlined apply to the titration of bases with strong acids, but, of course, with the direction of pH change reversed and with the end points tending to lie on the acid side of pH 7.0. A hydrogen ion concentration of $10^{-7}N$ or pH 7.0 is called the neutral point because it is the concentration of both the hydrogen and the hydroxyl ions in pure water; but it is evidently seldom the practical or even the theoretical point of neutrality for titrations.

As phenolphthalein is the more generally useful indicator for the titration of acids with strong bases so methyl red is the more generally useful indicator in the titration of bases with strong acids. Each fails, however, when the acid or base is very weak, and each may be replaced by a more suitable indicator in special cases. For the treatment of these cases the reader should consult the detailed description of the theory of titration in one of the papers mentioned above.

Where high color or turbidity interferes with the use of indicators in titration the hydrogen electrode is often useful. See Böttger (1897), Hildebrand (1913) Michaelis (1917). Since it may be necessary only to detect the break in the titration curve, the hydrogen electrode system and potentiometer system used for this purpose may be very simple. The hydrogen electrode has the advantage that it may often be used where colorimetric tests are impracticable and that it may be linked electrically with automatic regulating and recording instruments such as Leeds and Northrup Company have devised for industrial use.

Pinkhof (1919) has suggested special half-cells with single potentials equal to those of the end points of titrations, thereby eliminating the necessity of a potentiometer. A galvanometer or electrometer indicates equalization of potentials and hence the attainment of the "end point."

Since volumetric determinations of acids and bases involve one or the other method of determining some pH value, the understanding of the principles involved is essential to the intelligent

interpretation of data in the titration of those natural products which can be shown to be not susceptible to the exact treatment developed for the titration of strong acids and bases.

In the majority of cases the titration of such solutions reduces to a mere revelation of differences in total buffer action and furnishes but one point on the titration curve. The procedure often followed is comparable with the practice of the ancient Romans who, according to Trillat (1916), (cf. Stephanides 1916) titrated natural waters with drops of red wine. While modern standards of concentration are more exact than the wine standard of the Romans their significance is largely lost by a choice of indicators as accidental as the Roman choice of the coloring matter of red wine. The frank admission that the content of acids in some complex solutions cannot be determined by titration need not destroy the value of the information gained by a titration if this information be correctly used. But too often the matter is carried to an extreme. In the routine methods for titrating milk a perfectly simple test has been elaborated until it not only has become confusing to a chemist but so misleading to the creamery man that it is causing large economic losses. Often the initial pH of a solution is of greater significance than is the titration value obtained after juggling the solution with acid or alkali. Illustrations of this are to be found in the author's treatment of bacteriological culture media (Clark, 1915).

Having followed some of the salient features of titration and found this procedure linked with the more general aspects of hydrogen ion determinations the reader is reminded of those relations among acids and bases outlined in Chapter I which point to certain:—

GENERAL CONSIDERATIONS. As a comprehensive generalization it may be said that the hydrogen ion concentration of a solution influences in some degree every substance with acidic or basic properties. When we have said this we have said that the hydrogen ion concentration influences the great majority of compounds, especially those of biochemical interest. Such a generalization, however, would be misleading if not tempered by a proper appreciation of proportion. Rarely is it necessary to consider the ionization of the sugars since their dissociation constants are of the order of 10^{-13} and their ionization may be generally neglected in the pH region usually encountered in physiological studies. Likewise there are zones of pH within which any given acidic or basic group will be found in dilute solution to be in a practically undissociated or fully dissociated state. Perhaps there is no more vivid way of illustrating this than by a contemplation of the conduct of indi-

cators. Above a certain zone of hydrogen ion concentration phenolphthalein solutions are colorless. Below this zone (until intense alkalinity is reached) only the colored form exists. Within the zone the *virage* of a phenolphthalein solution is intimately related to the hydrogen ion concentration. The conduct of phenolphthalein, which happens to be visible because of tautomeric changes which accompany dissociation, is a prototype of the conduct of all acids. Just as we may suppress the dissociation of phenolphthalein by raising the hydrogen ion concentration of the solution so may we suppress the dissociation of any acid if we can find a more intensely ionizing acid with which to increase the hydrogen ion concentration of the solution. Similar relations hold for bases, and, if we regard methyl red as a base, we may illustrate with it the conduct of a base as we illustrated the conduct of an acid by means of phenolphthalein.

Such illustrations may serve to emphasize the reason underlying the following conclusion. Whenever, in the study of a physiological process, of a step in analysis requiring pH adjustments or of any case involving equilibria comparable with those mentioned above, there is sought the effect of the pH of the solution, it may be expected that no particularly profound effect will be observed beyond a certain zone of pH. Within or at the borders of such a zone the larger effects will be observed. From this we may conclude that the methods of determining hydrogen ion concentrations should meet two classes of requirements. In the first place, when the phenomenon under investigation or control involves an equilibrium which is seriously affected by the pH of the solution, the method of determining pH values should be the most accurate available. In the second place, when the equilibrium is held practically constant over a wide range of pH, an approximate determination of pH is sufficient and refinement may be only a waste of time.

Neglecting certain considerations which often have to enter into a choice of methods it may be said that the electrometric method had best be applied in the first case and the indicator method in the second. When the nature of the process is not known, and it therefore becomes impossible to tell *a priori* which method is to be chosen, the colorimetric method becomes a means of exploration and the electrometric method a means of confirmation.

Exception will be taken to this statement as a comprehensive one for there are cases where one or the other method has to be discarded because of the nature of the solution under examination. Nevertheless, in general, the utility of the colorimetric method lies in its availability where approximations are needed and exact determinations are useless and also in its value for reconnaissance; while the value of the electrometric method lies in its relative precision.

In some instances the qualitative and quantitative relations of a phenomenon to pH should be carefully distinguished. Note, for instance, the significance of an optimum or characterizing point. Consider the conduct of phenol red and of cresol red. These two indicators appear to a casual observer to be very much alike in color and each exhibits a similar *virage* in buffer solutions of pH 7.6, 7.8, etc. Careful study, however, shows that each point on the dissociation curve of phenol red lies at a lower pH than the corresponding point on the dissociation curve of cresol red. If the half transformation point be taken as characteristic it may be used to *indentify* these two indicators. Likewise it is the *dissociation constant* of an acid or a base, the *isoelectric point* of a protein, the *optimum pH* for acid agglutination of bacteria, or an optimum for a process such as enzyme activity that furnishes *characteristic* data.

When a correlation is observed between pH and some effect, the mere determination of pH alone will of course throw but little light upon the real nature of the phenomenon except in rare instances. Determination of the hydrogen ion concentration will not even distinguish whether a given effect is influenced by the hydrogen or the hydroxyl ions, nor will it always reveal whether the influence observed is direct or indirect. It is true, however, that, even when the hydrogen ion concentration is effective through remote channels, it may be very important. Therefore advantage should be taken of the comparative ease with which the concentration of hydrogen ions may be determined or controlled and their influence known or made a constant during the study of any other factor which may influence a process. From this point of view methods of determining hydrogen ion concentration take their place beside thermometers, and buffer mixtures beside thermostats.

Living cells are dependent upon the maintenance of a strictly limited hydrogen ion concentration in their environment. The recognition of this as a fact, independently of any theory whatever regarding the channels of influence, has brought hydrogen ion methods into the culture laboratory and into the garden. Accustomed as we are to dealing with ponderable quantities of material we are sometimes startled by the fact that a cell is dependent upon the maintenance of an environment varying between the limits 0.000,001 and 0.000,000,01 gram hydrogen ions per liter. Sometimes the permissible limits are even closer but the order of magnitude remains the same. Such values,¹ however, do not represent entities separable from the other material present in solution. They represent only a position of balance among relatively *large quantities* of material containing a reserve of potential hydrogen ions.

Still there does remain the fact that such minute and determinable quantities indicate the position of equilibrium among important substances and serve to orient relations unsuspected by biochemists before the development of the theory of electrolytic dissociation. It may now fairly be asked whether there are other relations of importance to the delicate adjustments of life processes to which quantities of the magnitude mentioned above may be an index. If we survey the biochemical activity of the hydrogen ions it appears that the hydrogen ion functions chiefly as a conditioning agent. It is only indirectly concerned with the chemical transformations which are the more intimately connected with life processes. Some of these chemical transformations directly, others indirectly, are capable of being resolved into oxidation-reduction processes. These in many instances amount to nothing more or less than transfers of electrons and in other instances to electron transfers which are accompanied by rearrangements. Philosophically considered there must be a period of freedom for an electron sometime in its transfer. Practically, free electrons have only been detected in such solutions as those of liquid ammonia. Nevertheless an electrode in a ferric-ferrous solution indicates the transfer pressure of the electrons. It remains to be seen if the

¹ The mensurability of such quantities is due to the magnitude of the electrical charge carried by each hydrogen ion and to the fact that at 10^{-7} N there are still in solution about 10^{16} hydrogen ions per litre.

methods which have been applied in this last instance have any great significance for biochemistry. That they have significance in the study of reduction by bacteria is indicated by preliminary work of Gillespie (1920) and of Clark (1920).

ANALYSES. The empiricism that characterized the development of analytical methods in the hands of Fresenius and others left specifications for the use of mixtures of acids, such as acetic, and their alkaline salts in many separations. This we now know controls the hydrogen ion concentration. Here and there in the special literature are to be found the calculated hydrogen ion concentrations in such cases and in other cases directions which are somewhat more precise than the customary "slightly acid" or "slightly alkaline." More recently there has been undertaken direct experimentation with hydrogen electrode or indicator methods. The need of further development was voiced some years ago by Dr. Hillebrand of the Bureau of Standards when he indicated to the Washington Chemical Society the need of a systematic investigation of all analytical methods. One type of information urgently needed may be learned from the papers of Blum, of Fales and Ware and of Hildebrand. Colorimetric pH measurements on carbonate equilibria are furnishing valuable information in several simple analytical methods. Kolthoff is working on the relation of pH to certain oxidation-reduction titrations. Many qualitative color reactions remain to be studied.

References. Böttger (1897), Brönsted (1911), Blum (1913, 1914, 1916), Eastman-Hildebrand (1914), Fales-Ware (1919), Garard-Sherman (1918), Haas (1916), Hildebrand (1913), Hildebrand-Bowers (1916), Kober-Sugiura (1913), Kolthoff (1919-20), Koritschoner-Morgenstern (1919), Marriott (1916), Oettingen (1900), Osterhout (1918), Robinson (1919), Levy-Cullen (1920), Liempt (1920), Swanson-Tague (1919), Tague (1920), Zoller (1920).

AUTOLYSIS of tissue is governed by the activity of enzymes which are sensitive to the concentration of hydrogen ions. As the resultant of the activity of two types of enzymes (Dernby) autolysis is controlled by the pH which brings into play the activity of each.

References. Bradley (1916), Bradley-Taylor (1916), Dernby (1917-1918), Morse (1916-1917).

BACTERIOLOGY. A review of the applications in bacteriology up to 1917 is given by Clark and Lubs (1917).

Adjustment of the reaction of media by the old titrimetric procedure was criticised by Clark (1915), and, on the introduction of suitable indicators and the evidence for the advantage of adjusting on the pH basis, the titrimetric method has been abandoned for more significant and easier modern methods. Studies on growth optima (which see below) have shown that for the cultivation of most saprophytes approximate indicator control without the use of standards is sufficient (see Chapter VII). For special purposes and especially for the study of certain important pathogens it is well to adjust with the precision attained with standards. Seldom is electrometric control necessary.

References. Bovie (1915), Clark (1915), Clark-Lubs (1916, 1917), Conn (1919), Fennel-Fisher (1919), Henderson-Webster (1907), Hurwitz-Meyer-Ostenberg (1915-1916), Jones (1919), Kligler (1917), Kligler-Defandorf (1918), Norton (1919).

Acid agglutination of bacteria, first definitely recognized by Michaelis (1911) in its relation to hydrogen ion concentration, has been found to be of some diagnostic use in the study of the typhoid-para typhoid group of bacteria, and has aided the resolution of the different types of pneumococci (Gillespie, 1914). The discovery by Arkwright of separately agglutinable constituents opened up some investigations of possibly wide bearing. Buchanan has indicated some of the possible relations to sera agglutination.

References. Arkwright (1914), Barendrecht (1901), Bechhold (1904), Beintker (1912), Beniasch (1912), Bergey (1912), Bondorf (1917), Buchanan (1919), Eisenberg (1919) (contains review and bibliography), Field-Teague (1907), Georgi (1919) Gieszczykiewicz (1916), Gillespie (1914), Grote (1913-1914), Heimann (1913), Jaffé (1912), Kemper (1916), Krumwiede-Pratt (1913), Markl (1915), Michaelis (1911, 1915, 1917), Michaelis-Adler (1914), Murray (1918), Poppe (1912), Radsma (1919), Schidorsky-Reim (1912), Sears (1913), Sgalitzer (1913), Tulloch (1914).

Disinfectant action of acids and bases is certainly in large measure a function of hydrogen or hydroxyl ion concentration; but specific effects of certain acids and bases, which were suspected before, have now been more clearly demonstrated by the use of

hydrogen ion methods. With the conductivity method Winslow and Lochridge were able to show the effect of the hydrogen ion in simple solutions and predicted relations which more powerful methods have extended to complex media. There is evidence that the more direct action of the hydrogen ion upon cells must be distinguished from its control upon the effective state of a toxic compound. Each of these aspects is of importance in the study of relative disinfectant powers of antiseptics by procedures such as the Rideal-Walker test (Wright). See suggestive material in Dakin and Dunham (1917). Closely allied with the subject is the influence of the hydrogen ion on thermal death rates. In the pasteurization and sterilization of various products economies may be effected by taking into consideration the fact that lower temperatures suffice for very acid products. It is interesting to note that the hydrogen ion considered as an entity rather than in its relation to equilibria has been described as the most toxic of all ions; while from the point of view of equilibria its activity must be maintained at a definite level to preserve life.

References. Bial (1902), Browning-Gulbrandsen-Kennaway (1919), Clark, J. F. (1899), Clark-Lubs (1917), Cohen-Clark (1919), Friedenthal (1919), Krönig-Paul (1897), Norton-Hsu (1916), Paul-Birstein-Reuss (1910), Paul-Krönig (1896), Waterman (1915), Winslow-Lochridge (1906), Wright (1917).

Growth optima is a subject which has received its greatest attention since the revelation of the mistakes to which the old procedure of adjusting media led. While it has been shown that many bacteria flourish in the initial stages of a culture even though the pH be varied within wide limits (Cohen-Clark) others require more restricted initial conditions. A distinction must be made between the fermentative and the growth conditions. Limitations imposed by the reaction of natural environments are of great importance. (See soils.)

References. Allen (1919), Bunker (1919), Cohen-Clark (1919), Cole-Lloyd (1917), Dernby-Avery (1918), Gainey (1918), Gillespie (1918), Gillespie-Hurst (1918), Hägglund (1915), Kligler (1918), Lazarus (1908), Lord (1919), Lord-Nye (1919), Lloyd (1916), Meyerhof (1916), Schoenholz-Meyer (1919), Shohl-Janney (1917), Svanberg (1919), Wright (1917).

The influence of pH upon the production of specific products and upon types of bacterial metabolism has only begun to be studied. Partial control of the production of diphtheria toxin (Bunker, Davis) has created considerable interest. The author has found that not only the activity but the production of gelatinase by *Proteus* is under the partial control of the pH of the medium. Investigations on yeast fermentations are being pursued by Euler and his colleagues. Some important modifications of yeast fermentation in alkaline media, studied during the war, are appearing in the literature. The details will be watched for with interest.

References. Atkin (1911), Boas (1919), Bronfenbrenner-Schlesinger (1918), Bunker (1919), Davis (1918), Euler-Blix (1919), Euler-Emberg (1919), Euler-Hammarsten (1916), Euler-Svanberg (1917) (1919), Green (1918), Gröer (1912), Itano (1916), Jacoby (1918), Lord-Nye (1919), Northrup-Ashe-Senior (1919), Sasaki (1917), Venn (1920), Wyeth (1919).

The pH limits of growth and general metabolism have naturally been the chief interest of those making the first surveys of the influence of hydrogen ion concentration upon bacterial activity. The first clear definition of the problem was published by Michaelis and Marcora in 1912 and was followed by more extensive investigations by the author. The self limitation of acid fermentations, if not precisely defined in terms of pH (Clark, 1915; Wyeth, 1918, 1919, Van Dam, 1918), has certain practical applications (Clark, 1915) which are of importance. Certain features of the phenomena were developed in the differential test known as the methyl red test (Clark and Lubs 1915, 1917). A similar test for the separation of streptococci has been developed by Avery and Cullen from Ayers' (1916) discovery of different pH limits for different groups. pH limits for special organisms which have become of commercial significance are found in the control of "rope" in bread (Cohn-Walbach-Henderson-Cathcart) and "scab" on potatoes (Gillespie-Hurst).

References. Avery-Cullen (1919), Ayers (1916), Ayers-Johnson-Davis (1918), Barthel (1918), Barthel-Sandberg (1919), Boas-Leberle (1918), Bunker (1919), Clark (1915, 1916, 1917, 1918), Clark-Lubs (1915-1917), Cohen-Clark (1919), Cohn-Walbach-Henderson-Cathcart (1918), Cole-Onslow (1916), Cullen-Chesney (1918), Currie (1917), Van Dam (1918), Duggar-Severy-Schmitz

(1917), Euler-Emberg (1919), Evans (1918), Fred-Davenport (1918), Frothingham (1917-1918), Gates (1919), Gillespie-Hurst (1918), Grace-Highberger (1920), Hägglund (1915), Henderson (1918), Itano (1916), Itano-Neill (1919), Johannessohn (1912), Jones (1920), Kohman (1919), Kniep (1906), Lord (1919), Lüers (1914), Meacham (1918), Michaelis-Marcora (1912), Shaw-Mackenzie (1918), Svanberg (1918), Waksman (1918), Waksman-Joffe (1920), Wyeth (1918-1919), Winslow-Kligler-Rothberg (1919), Wolf (1918), Wolf-Harris (1917), Wolf-Telfer (1917).

BEER. As originally outlined by Pasteur the "reaction" of wort has much to do with the brewing of beer. The control of "disease" and of the protein material held in solution is to some extent dependent upon pH as is the activity of the enzymes concerned at each stage.

References. Adler (1915, 1916), Emslander (1914-1919), Leberle-Lüers (1914), Lüers (1914), Lüers-Adler (1915), Schjerning (1913). See also bacteriology, enzymes and proteins.

BLOOD. The hydrogen ion concentration of blood is regulated with remarkable constancy. The mechanism immediately concerned in maintaining this constancy is the buffer action of the carbonate supplemented by the buffer action of the protein and the phosphate and influenced by the heterogeneous equilibria between the solid and liquid phases of the blood.

Following Höber (1903) there have been a great many gas chain measurements of the hydrogen ion concentration of the blood under various pathological, environmental and dietary conditions. One of the outstanding results has been the establishment of the fact that the pH itself varies but slightly from about 7.5.

From one point of view the blood may be regarded as a scavenger, burning the waste products in the tissues it perfuses, and carrying off the final products of combustion of which CO_2 is one of the most important for the acid base equilibria under consideration. Under a given content of buffer in the blood the hydrogen ion concentration would be maintained constant under this inflow of CO_2 by the maintenance of a constant CO_2 pressure in the lungs; but under varying buffer content the hydrogen ion concentration could only be maintained constant by a mechanism directly responsive to hydrogen ion concentration and capa-

ble of altering the CO_2 pressure. It seems that the respiratory centre is thus directly responsive to the hydrogen ion concentration and by its regulation of the breathing maintains in the alveolar air that level of CO_2 pressure which is in harmony with the equilibria centered about constant pH under varying conditions. Of this Haldane says: "The respiratory centre is enormously more delicate as an index of change in hydrogen ion concentration of the blood than any existing physical or chemical method." Clinical methods based on the measurement of the alveolar CO_2 tension are now extensively used (see Van Slyke). On the other hand, the CO_2 tension is but one item of a complicated set of equilibria. It often becomes of importance to know the relative proportions of the other constituents of the acid base equilibria. In pathological conditions the oxidative processes may be at fault and the carbonate equilibria must be adjusted to accommodate the products of incomplete combustion in the effort of the body to maintain constant hydrogen ion concentration in the blood. Therefore it becomes important to learn the relation of the CO_2 content to the alkaline reserve. When this is done by gas chain or indicator titrations the hydrogen electrode and indicator methods again enter the subject from which they were to some extent displaced when it was found that there was no particular object in studying a constant maintained physiologically with a degree of precision often beyond the precision of experimental measurement.

Intimately connected with the regulation of the hydrogen ion concentration of the blood are the functions of the kidneys (see Cushny). By their action there are eliminated the nonvolatile products of metabolism, several of which are of great importance for the acid base equilibria of the blood. The colorimetric determination of the pH of the urine is a comparatively simple procedure which furnishes valuable data when properly connected with other data. (See for instance Blatherwick, and the works of Henderson, of Palmer and of Van Slyke.)

Recently interest is centering upon the mutual relation between the hydrogen ion concentration of the blood and the state of the oxidized and reduced respiratory pigment. It appears that oxyhaemoglobin contains a stronger acid group than reduced haemoglobin. It follows at once that oxidation or reduction can

shift the acid base equilibria and conversely that a shift in these equilibria can affect the oxidizing power of the blood.

While the greatest interest has centered in the subjects briefly mentioned above, there remain innumerable other problems of importance. Of these there may be mentioned the relation of the pH of the blood to the calcium-carrying power, to the activity of various enzymes, to the permeabilities of tissue membranes, to the activity of leucocytes, and to various reactions used in the serum diagnosis of disease.

On very short notice Dr. Glenn Cullen has kindly suggested the references immediately following, which will familiarize the student with the main aspects of some of the subjects noted in this section. The remaining references are offered with the caution that the list is far from complete and undoubtedly unbalanced. They are the references which have fallen into the author's bibliography during the years that he has maintained an interest in matters bearing upon the subject of this book.

The more important fundamental aspects of neutrality regulation in the blood are set forth by L. J. Henderson in *Das Gleichgewicht zwischen Basen und Säuren im tierischen Organismus*, *Ergeb. Physiol.* 8, 254 (1909). In the acidosis of diabetes the relation of the neutrality regulating mechanism to the introduction into the body fluids of abnormal acids is set forth in the papers by Palmer and Henderson (1913), Palmer and Van Slyke (1917), Hasselbalch and Gammeltoft (1915), and Van Slyke and coworkers.

Other important references on acidosis are Cullen (1917), Hasselbalch (1917), Hasselbalch and Gammeltoft (1915), Howland and Marriott (1916), Michaelis (1914), Milory (1915), Naunyn (1906), Newburgh-Palmer-Henderson (1913), Palmer and Henderson (1913), Palmer and Van Slyke (1917), Peabody (1914), Stillman-Van Slyke-Cullen-Fitz (1917), Van Slyke (1917), Van Slyke-Cullen (1917).

Papers bearing particularly upon the respiration phase of the subject are:—Bayliss (1918), Hasselbalch (1912, 1916, 1917), Hasselbalch-Lundsgaard (1912), Henderson (1908, 1909, 1920), Michaelis-Rona (1909), Parsons (1917, 1919, 1920), Scott (1917). Barcroft (1914) summarizes the older work.

Acidosis and shock is treated in the 1918 report (No. 7) of the British Medical Research Committee.

References. Abel-Fürth (1916), Adler-Blake (1911), Aggaz-zoti (1907), Auerbach-Friedenthal (1903), Barcroft (1914), Bayliss (1918), Begun-Münzer (1915), Benedict (1906), Bienstock-Czáki (1917), Blatherwick (1914), Bottazzi (1911), Bugarszky (1897), Bugarszky-Tangl (1898), Corral (1915), Cullen (1917), Debenham-Poulton (1918), Donegan-Parsons (1919), Elias-Kolb (1913), Farcas (1903), Farcas-Schipiades (1903), Fitz-VanSlyke (1917), Foà (1905), Fraenckel (1903), Friedenthal (1902, 1903, 1904), Gettler-Baker (1916), Haldane (1916), Haskins (1919), Hasselbalch (1912, 1920), Hasselbalch-Gammeltoft (1915), Hasselbalch-Lindhard (1911, 1916), Hasselbalch-Lundsgaard (1912), Hasselbalch-Warburg (1918), Henderson (1908, 1920), Henderson-Palmer (1913, 1915), Henderson-Spiro (1908), Höber (1900, 1902, 1910), Höber-Jankowsky (1903), Hooker-Wilson-Connet (1917), Howland-Marriott (1916), Howe-Hawk (1914), Hurwitz-Lucas (1916), Irwin (1919), Isaacs (1917), Kelly (1915), Konikoff (1913), Kreibich (1910), Levy-Rowntree-Marriott (1915), Levy-Rowntree (1916), Löb (1911), Lundsgaard (1912), Marriott (1916), McClendon (1916), McClendon-Magoon (1916), McClendon-Sedlov-Thomson (1917), McClendon-vonMeyesenbug-Engstrand-King (1919), Macleod (1916, 1918, 1919), Macleod-Knapp (1918), Masel (1913), Menton-Crile (1915), Michaelis (1914), Michaelis-Davidoff (1912), Michaelis-Rona (1909), Milroy (1915, 1917), Momase (1915), Newburgh-Palmer-Henderson (1913), Palmer-Henderson (1913, 1915), Palmér-VanSlyke (1917), Parsons (1917, 1919, 1920), Peabody (1914), Peters (1914, 1917), Pfaundler (1905), Palányi (1911), Poulton (1915), Quagliariello-Agostino (1912), Quagliariello (1912), Reemlin-Isaacs (1916), Rhorer (1901), Ringer (1909), Robertson (1909, 1910), Rolly (1912, 1914), Rona-György (1913), Rona-Takahashi (1913), Rona-Ylppö (1916), Salge (1912, 1913), Schwartz-Lemberger (1911), Scott (1916, 1917), Sellards (1912), Skramlik (1911), Snapper (1913), Sonne-Jarlöv (1918), Spiro-Henderson (1908), Spiro-Pemsel (1898), Stillman-Van Slyke-Cullen-Fitz (1917), Straub-Meier (1918), Szili (1906, 1909), Van Slyke D. D. (1917), Van Slyke-Cullen (1917), Van Slyke-Palmer (1919), Van Slyke-Stillman-Cullen (1917, 1919), Wilson-Stearns-Thurlow (1915), Winterstein (1911, 1915), Ylppö (1916), Zunz (1918).

BREAD. In the baking of bread it is essential that the proteins, such as gluten, which are responsible for the holding of the gas, shall be conditioned by the proper pH. The pH may also control the growth of the "rope" organism. The activity of yeast and the evolution of CO_2 from baking powders have relations to the pH of the dough.

References. Cohn-Cathcart-Henderson (1918), Cohn-Henderson (1918), Cohn-Walbach-Henderson-Cathcart (1918), Henderson (1918), Henderson-Cohn-Cathcart-Wachman-Fenn (1919), Henderson-Fenn-Cohn (1919), Jessen-Hansen (1911), Landenberger-Morse (1918), Lüers (1919), Wahl (1916).

BODY FLUIDS (other than blood, urine, digestive juices, cerebrospinal fluid).

References. Collip (1920), Farkas-Scipiadès (1903), Foà (1905, (1906), Fraenckel (1905), Gies (1916), Goldberger (1917), Löb-Higuchi (1910), Long-Fenger (1915, 1916), Marshall (1915), Michaelis-Kramsztyk (1914), Okada (1915), Quagliariello (1916), Shepard-Gies (1916), Uyeno (1919).

BOTANY. See plant distribution and soils.

References. Clevenger (1919), Haas (1916), Hempel (1917), Hoagland (1917, 1918, 1919), Kappen (1918), Loew (1903), Wagner (1916), Wherry (1916, 1918, 1919).

BUFFER MIXTURES. See text, also Enklaar (1912), Fels (1904), Friedenthal (1904), Michaelis (1910), Prideaux (1911, 1916, 1917), Ringer (1909), Schmidt-Finger (1908), Walpole (1914) and consult tables (Scudder 1914) of dissociation constants as directed in Chapter I for the selection of mixtures of acids or bases and their salts.

CARBONATE equilibria are undoubtedly the most important of all equilibria in which the hydrogen ion concentration is concerned. They are the chief equilibria concerned in the regulation of the hydrogen ion concentration of the blood, sea water and many of the natural solutions resulting from the activity of or nourishing cells of various types. Their biological significance has been entertainingly described by Henderson in his *The Fitness of the Environment*. Because of the biological importance of the subject the study of carbonate equilibria has been pursued actively by biochemists and many details will be found among the papers on such subjects as blood. One of the most extensive experimental

studies was that of Auerbach and Pick. Johnston has set forth several aspects of the subject which are of particular interest to the geologist and the water analyst. See "water," "blood," "analyses."

References. Auerbach-Pick (1912), Frary-Nietz (1915), Henderson (1913), Henderson-Black (1908), Johnston (1916), Johnston-Williamson (1916), McClendon (1917), McClendon-Shedlov-Thomson (1917), Michaelis-Rona (1914), Prideaux (1915), Seyler-Lloyd (1917), Thiel-Stroheker (1914), Walker-Cormack (1900), Van Slyke (1917).

CATALYSIS. The catalytic activity of the hydrogen and the hydroxyl ions in such transformations as the hydrolysis of cane sugar has taken a prominent place in the development of the theory of electrolytic dissociation. Under limited conditions one or another of these catalytic processes is proportional to the concentration of the hydrogen or the hydroxyl ions; but there may enter the action of neutral salts, or as shown by Senter, by Acree and by others, the activity of the undissociated portions of acids or bases.

The literature on hydrogen and hydroxyl ion catalyses is completely reviewed in *Die Rolle der Katalyse in der Analytischen Chemie* by Gertrud Woker, Enke, Stuttgart, 1910, 1915.

CATAPHORESIS. See references under isoelectric point. Michaelis (1914), Svedberg-Anderson (1919).

CEREBROSPINAL FLUID, NERVES, ETC.

References. Bisgaard (1913), Bottazzi-Craifaleanu (1916), Chiò (1907), Collip (1920), Felton-Hussey-Bayne-Jones (1917), Hurwitz-Tranter (1916), Levinson (1917, 1919), Mayer (1916), Moore (1917), Weston (1916).

CHEESE.

References. Allemann (1912), Barthel-Sandberg (1919), Van Dam (1910).

COLLOIDS. That the dispersion of colloids may be influenced by the "reaction" of the medium has long been known. So widely scattered is the literature on this particular phase of colloid chemistry that the author has made no attempt to assemble it except through the special literature on proteins. It is through the study of protein solutions that the most distinctive advances have been made. Beginning with Hardy the study of proteins as amphoteric electrolytes has been carried forward by Pauli, Michaelis,

Robertson, Sørensen, Henderson, Loeb and others until there has developed a distinct protest against the separation of *certain* of the phenomena of colloids from the application of the simpler relations of crystalloids. How far the matter may be pushed in its application to other types of material taking the "colloidal state" remains to be determined.

A very good discussion of the relation of the developments in protein chemistry to colloid chemistry is given by Sørensen (1917). (Compare Loeb, 1919.)

References. Clowes (1913), Ellis (1911), Lachs-Michaelis (1911), Lillie (1907), McBain-Salmon (1920), Michaelis-Rona (1919), Ostwald (1912), Rona-Michaelis (1919), Smith (1920), Walpole (1914).

COMPARATIVE AND GENERAL PHYSIOLOGY.

References. Aggazzotti (1913), Andrus (1919), Bethe (1909), Cohn (1917), Cremer (1906), Crozier (1915, 1916, 1918, 1919), Dale-Thacker (1914), Fletcher-Hopkins (1907), Goldberger (1917), Harvey, E. N. (1920), Harvey, R. B. (1920), Herbst (1904), Hiruma (1917), Höber (1910), Hurwitz (1910), Jacobs (1920), Jewell (1920), Kahlenberg (1900), Kopaczewski (1914), Křiženecký (1916), Loeb (1898, 1903, 1904, 1906), Loeb-Wasteneys (1911), Lloyd (1916), McClendon (1916, 1920), McClendon-Mitchell (1912), Mines (1912), Moore (1919), Moore-Roaf-Whitney (1905), Meyerhof (1918), Neugarten (1919), Oden (1916), Parnas-Wagner (1914), Pechstein (1915), Porcelli-Titone (1914), Popielski (1919), Resch (1917), Richards (1898), Roaf (1912), Rona Wilenko (1914), Roth (1917), Schwyzer (1914), Shelford-Powers (1915), Shohl (1914), Straub-Meier (1919), Warburg (1910), Wells (1915), Whitley (1905).

CRYSTALLOGRAPHY. Wherry (private communication) states that there is reason to believe that the pH of a medium may sometimes control crystal form.

DAKIN'S SOLUTION.

Reference. Cullen-Austin (1918).

DIGESTIVE SYSTEM. The digestive tract is primarily the channel for the intense activity of hydrolytic enzymes and as such is provided with mechanisms for the establishment of hydrogen ion concentrations favorable to these enzymes. Hydrogen electrode methods have correlated the regional activity of particular en-

zymes with the reactions there found, have clarified some of the differences between the digestive processes of infancy and adult life, aided in the explanation of the acid and alkali formation, and have been of service in the improvement of clinical methods for the assay of pepsin activity and the diagnosis of abnormal secretion of hydrochloric acid in the stomach. The control of specific physiological functions such as secretion of conditioning agents (see Bayliss, 1918), permeabilities, and activities of the varied musculature, as well as investigations upon the condition in the digestive tract of substances such as calcium and phosphate which form insoluble precipitates are subjects which present promising material for the application of modern methods. Shohl (1920) has recently reviewed and improved methods of studying gastric acidity.

References. Allaria (1908), Ambard-Foà (1905), Auerbach-Pick (1912, 1913), Cannon (1907), Christiansen (1911, 1912), Davidsohn (1911, 1912, 1913), Foà (1905, 1906), Fowler-Bergeim-Hawk (1915), Fraenkel (1905), Graham (1911), Hahn (1914), Hess (1915), Howe-Hawk (1912), Huenekens (1914), Krummacher (1914), Long-Fenger (1917), McClendon (1915), McClendon-Myers-Culligan-Gydesen (1919), McClendon-Shedlov-Thomson (1917), McClendon-Shedlov-Karpman (1918), McWhorter (1918), Menten (1915), Michaelis (1917), Michaelis-Davidsohn (1910), Michaelis (1918), Myers-McClendon (1920), Nelson-Williams (1916), Popielski (1919), Rolph (1915), Rona-Neukirch (1912), Salge (1912), Schryver-Singer (1913), Shohl (1920), Tangl (1906), Ylppö (1916).

DISSOCIATION CONSTANTS as determined with the hydrogen electrode or indicator methods. Compare Chapter I.

References. Agostino-Quagliariello (1912), Dernby (1916), Eijlman (1906), Kastle (1905), Kolthoff (1918), Michaelis (1911, 1913, 1914), Michaelis-Garbendia (1914), Michaelis-Rona (1913, 1914), Prideaux (1911), Salm (1906, 1908), Scudder (1914), Tizard (1910), Weisse-Meyer Levy (1916). See Indicator constants.

DRY CELLS.

Reference. Holler-Ritchie (1920).

ELECTROPLATING.

References. Bennett-Rose-Tinkler (1915), Blum (1920).

ENZYMES. The activity of enzymes as influenced by the hydrogen ion concentration of the solution has occupied the atten-

tion of many investigators since the publication of Sørensen's paper (1909). The analogy between the activity curves of several enzymes and the curves relating the "dissociation residues" of amphoteric electrolytes to pH suggested to Michaelis the amphoteric nature of enzymes (cf. Loeb 1909). Holderer's observations on the extraction of enzymes from cells with solvents of different reaction are most suggestive. The necessity of controlling the pH of enzyme solutions for assays as well as in the study of the effect of salts and in experiments having to do with the formulation of the laws of enzyme activity (Van Slyke and Cullen) is now generally recognized. Barendrecht in the development of his radiation theory notes the special importance of the hydrogen ions.

The following is a rough classification of studies on specific enzymes.

Amylase. Falk-McGuire-Blount (1919), McGuire-Falk (1920), Sherman-Thomas-Baldwin (1918, 1919), Sherman-Schlessinger (1915), Sherman-Thomas (1915), Sherman-Walker (1917).

Bacterial enzymes. Dernby (1917), Gröer (1912), Itano (1916), Kanitz (1903), Lord (1919), Meyer (1911), Waksman (1918).

Carboxylase. Neuberg (1915).

Catalase. Bodansky (1919), Euler-Blix (1919), Falk-McGuire-Blount (1919), Michaelis-Pechstein (1913, 1914), Phragmén (1919), Senter (1905), Sørensen (1909), Waentig-Steche (1911).

Cellase. Bertrand-Holderer (1910).

"*Diastases*" (Important historical references) Fernbach (1906), Fernbach-Hubert (1900).

Filtration of. Holderer.

Glycogenase. Norris (1913).

Emulsin. Bayliss (1912), Vulquin (1910).

Erepsin. Euler (1907), Dernby (1916), Rona-Arnheim (1913).

Esterases (lipase). Baur (1909), Davidsohn (1912-1913), Falk, I. (1918), Falk, K. (1916), Hulton-Frankel (1917), Rona (1911), Rona-Bien (1914), Rona-Michaelis (1911).

Invertase. Bertrand-Rosenblatt-Rosenblatt (1912), Fales-Nelson (1915), Griffin-Nelson (1916), Hudson (1910), Hudson-Paine (1910), Kanitz (1911), Michaelis-Davidsohn (1911), Michaelis-Menten (1913), Michaelis-Pechstein (1914), Nelson-Griffin (1916), Nelson-Vosburgh (1917), Sørensen (1909).

Lactase. Davidsohn (1913).

Maltase. Adler (1916), Kopaczewski (1912, 1914, 1915), Michaelis-Rona (1913, 1914), Rona-Michaelis (1913).

Oxidases, etc. Bunzel (1915), Bunzell (1916, 1917), Menten (1919), Reed (1916), Rose-Kraybill-Rose (1920).

Optimum temperature. Compton (1915).

Papain. Frankel (1917).

Peroxidase. Bouma-Van Dam (1918).

Pepsin. Christiansen (1912), Van Dam (1915), Davidsohn (1912), Funk-Niemann (1910), Gies (1902), Loeb (1909), Michaelis (1918), Michaelis-Mendelsohn (1914), Northrop (1919, 1920), Okada (1916), Peckelharing-Ringer (1911), Ringer (1918), Rohonyi (1912), Sørensen (1909).

Phosphatase. Adler (1915).

Rennet. Allemann (1912), Van Dam (1908, 1909, 1912, 1915), Michaelis-Mendelsohn (1913), Funk-Niemann (1910), Milroy (1915), Thaysen (1915).

Salivary diastase (ptyalin). Cole (1903), Michaelis-Pechstein (1914), Ringer-Trigt (1912). See amylase.

Taka-diastase. Okada (1916).

Trypsin. Auerbach-Pick (1913), Kanitz (1902), Michaelis-Davidsohn (1911), Palitzsch-Walburn (1912), Robertson-Schmidt (1908).

Theory of action. Barendrecht (1920), Loeb (1909), Michaelis (1909, 1914), Michaelis-Davidsohn (1910, 1911), Rohonyi (1911), Van Slyke-Cullen (1914).

Urease. Barendrecht (1920), Onodera (1915), Van Slyke-Cullen (1914), Van Slyke-Zacharias (1914).

FECES. Howe-Hawk (1912), Ylppö (1916). See also "digestive system."

FILTRATION. Hydrogen ion concentration, through its influence upon the dispersion of certain colloids and upon the conditioning of filter material, may control the filterability of a substance. Holderer's thesis from Perrin's laboratory presents in admirable form many of the theoretical aspects of the subject. A republication of this rare thesis is desired. The subject is not only of considerable theoretical interest but also of great practical importance. Buffer control with indicator tests may in many instances facilitate filtrations upon an industrial as well as a laboratory scale.

References. Aubel-Colin (1915), Holderer (1909, 1910, 1911, 1912), Homer (1917), Loeb (1919), Schmidt (1914), Strada (1908).

FOODS, pH OF. The National Canners Laboratory has made a number of determinations of the pH of canned foods. See also references to fruit juices in Clark-Lubs (1917). See also "milk" "cheese" and McClendon-Sharp (1919). The influence of pH upon the stability of "vitamines" has not yet been systematically studied to the author's knowledge. But compare Harden-Zilva (1918) and Zilva (1919).

GEOLOGY. See "carbonate equilibria." Also address of Wherry, December, 1919, meeting Geological Society, Boston.

GLUCOSE, decomposition of, as influenced by pH.

References. Elias-Kolb (1913), Henderson (1911), Mathews-McGuigan (1907), Michaelis-Rona (1909-1912), Nef (1913), Rona-Arnheim (1913), Rona-Doblin (1911), Rona-Wilenko (1914). Also references in Woker.

HAEMOLYSIS.

References. Atkin (1911, 1914), Fühner-Neubaur (1907), Gros (1910), Hellens (1913), Jordan (1903), Kozawa (1914), Krogh (1909), Lagrange (1914), Michaelis-Skwirsky (1909), Michaelis-Takahashi (1910), Teague-Buxton (1907), Walbum (1914, 1915).

HYDROGEN ION EQUILIBRIA. The hydrogen electrode and indicators in the determination of affinity constants, free energy, effect of neutral salts, hydrolysis etc.

References. Bjerrum (1907, 1910), Chow (1920), Denham (1908), Eucken (1907), Ellis (1916), Ferguson (1916), Frary-Nietz (1915), Hardman-Lapworth (1911), Harned (1915, 1916), Heyrowsky (1920), Jahn (1900, 1901), Lewis (1908, 1912, 1913), Lewis-Brighton-Sebastian (1917), Lewis-Randall (1914), Linhart (1919), Loomis-Acree (1911), Loomis-Essex-Meacham (1917), Löwenherz (1896), Margaillan (1913), McBain-Coleman (1914), MacInnes (1919), Nernst (1889), Newbery (1914), Noyes-Ellis (1917), Noyes-Freed (1920), Tizard (1910), Tolman-Greathouse (1912). See also numerous references in Abegg-Auerbach-Luther.

INDICATORS, natural.

References. Bribaker (1914), Crozier (1916, 1918), Haas (1916), Pozzi-Escot (1913), Sacher (1910), Scheitz (1910), Stephanides (1916), Trillat (1916), Walbum (1913), Watson (1913). See also Perkin and Everest.

INDICATOR CONSTANTS. See Prideaux.

References. Clark-Lubs (1917), Gillespie (1920), Paulus-Hutchinson-Jones (1915), Rosenstein (1912), Schaeffer-Paulus-Jones (1915), Salm (1904), Tizard (1910).

ISOELECTRIC POINTS. See Chapter I.

References. Cohn-Gross-Johnson (1920), Michaelis (1911, 1912), Michaelis-Bien (1914), Michaelis-Davidsohn (1910, 1911, 1912, 1913), Michaelis-Grinoff (1912), Michaelis-Mostynski (1910), Michaelis-Pechstein (1912), Michaelis-Takahashi (1910), Rona-Michaelis (1910), Loeb (1918), Sørensen (1912, 1917).

MILK.

References. Allemann (1912), Aron (1914), Baker-Van Slyke (1919), Chapman (1908), Clark (1915), Cooledge-Wyant (1920), Van Dam (1908, 1918), Davidsohn (1912-1913), Foà (1905, 1906), Laqueur-Sachur (1903), Milroy (1915), Rona-Michaelis (1909), Sommer-Hart (1919), Stutterheim, Szili (1917), Taylor (1913), Terry (1919), Van Slyke-Baker (1918, 1919).

PERMEABILITY of cells.

References. Donnan (1911), Haas (1916), Harvey (1911, 1913), Holderer (1911), Lillie (1909), Odén (1916), Reemelin-Isaacs (1916), Snapper (1913), Stiles-Jorgensen (1915), compare filtration.

PHAGOCYTOSIS.

References. Hamberger-Heckma (1908), Koltzoff (1914), Sawtchenko-Aristovsky (1912).

PLANT DISTRIBUTION. Wherry, working with a simple field kit, has carried the sulfon phthalein indicators into the field and correlated the habitats of several plants with the pH of their soils. The information thus gained has aided in the cultivation of the blueberry and in the propagation of wild flowers hitherto uncommon or unknown to garden and greenhouse.

PROTEINS may be treated as amphoteric electrolytes (compare Chapter I). Increasing the hydrogen ion concentration of a solution of a basic salt of the protein tends to suppress the acidic dissociation and increase of the hydroxyl ion concentration of a solution of an acid salt tends to suppress the basic dissociation. When these suppressions reach a resultant maximum and the solution contains the maximum neutral protein the protein is said to be at its isoelectric point. The electrical charge upon the protein molecule which is thus induced to a large extent by the electroly-

tic dissociation affects the dispersion of the material and is intimately related to surface tension phenomena operative in precipitation and coagulation. If the solubility of a protein is very low while that of its salts is relatively high the protein may be precipitated at the isoelectric point. This principle is used in the commercial preparation of casein. Closely related to this is the adjustment of the pH of a solution for the crystallization of proteins.

Optima for denaturation of proteins by heat are to be distinguished from optima for coagulation or precipitation.

Denaturation by heat, and hydrolysis may alter the pH and the buffer action of protein solutions.

References. Agostino-Quagliariello (1912), Bugarszky-Liebermann (1898), Burrows-Cohn (1918), Chiari (1911), Chick (1913), Chick-Martin (1910, 1911, 1912, 1913), Cohn-Gross-Johnson (1920), Haas (1918), Handovsky (1910), Hardy (1899, 1905), Henderson-Cohn-Cathcart-Wachman-Fenn (1919), Henderson-Palmer-Newburgh (1914), Laqueur-Sackur (1903), Loeb (1918, 1919, 1920), Lloyd (1920), Manabe-Matula (1913), Michaelis (1909), Michaelis-Mostynski (1910), Michaelis-Rona (1910), Oryng-Pauli (1915), Patten-Johnson (1919), Pauli (1903, 1906, 1907), Pauli-Handovsky (1908, 1909, 1910), Pauli-Samec (1909, 1914), Pauli-Wagner (1910), Pechstein (1913), Procter-Wilson (1916), Quagliariello (1912), Resch (1917), Robertson (1907, 1909, 1910, 1918), Rohonyi (1912), Ryd (1917), Schmidt (1916), Schorr (1911), Sørensen (1917), Sørensen et al. (1917), Sørensen-Jürgensen (1911), Spiro (1904, 1913), Starke (1900), Ylppö (1913). See also Isoelectric point.

SEROLOGY. See also acid agglutination of bacteria, haemolysis, production of diphtheria toxin (Davis, Bunker), proteins, colloids.

References. Amako (1911), Atzler (1914), Buchanan (1919), Field-Teague (1907), Homer (1917, 1918), Landsteiner (1913), Lindenschatt (1913), Leschly (1916), Michaelis-Davidsohn (1912), Noguchi (1907), Tulloch (1914, 1918).

SOAP SOLUTIONS. McBain-Bolam (1918), McBain-Martin (1918), McBain-Salmon (1920).

SOIL ACIDITY has been confused by the complexities of titrimetric procedures, has been neglected, or has been considered to be

an unreality by one or another school. Gillespie (1916) obtained good agreement between pH values of soil extracts determined by means of the hydrogen electrode and again by means of indicators. The practical significance of this is now revealed by studies which show characteristic pH values for well-defined types of soil, which show correlations between the pH of soil extracts and the growth of beneficial or harmful microorganisms, and which show correlations between the natural distribution of plants and the pH of the soils in which they are found.

References. Blair-Prince (1920), Fischer (1914), Gainey (1918), Gillespie (1916, 1918), Gillespie-Hurst (1918), Hoagland (1917-1919), Hoagland-Christie (1918), Hoagland-Sharp (1918), Hudig-Sturm (1919), Joffe (1920), Kappen (1916), Knight (1920), Loew (1903), Morse (1918), Odén (1916), Plummer (1918), Rice-Osugi (1918), Saidel (1913), Salter-McIlvaine (1920), Sharp-Hoagland (1916, 1919), Stephenson (1919), Tijmstra (1917), Truog (1918), Truog-Meacham (1919), Wherry (1916, 1919, 1920).

SOLUBILITY. Examples of effect of pH on.

References. Böttger (1903), Ringer (1910). See proteins.

SURFACE TENSION.

References. Bottazzi-Agostino, Ellis (1911), Haber-Klemensiewicz (1909), Michaelis (1909), Schwyzer (1914), Willows-Hatschek (1919).

SWEAT.

References. Clark-Lubs, (1917), Talbert (1919).

TANNING.

References. Balderston (1913), Povarnin (1915), Sand-Law (1911), Thomas-Baldwin (1919), Wood-Sand-Law (1911).

TAUTOMERISM other than of indicators.

References. Biddle-Watson (1917), Fraenkel (1907), Nelson-Beegle (1919).

URINE. The excretion of acids and bases in the urine is one of the mechanisms by which the hydrogen ion concentration of the blood is preserved constant. For this reason the determination of the acid-base equilibria in the urine in their relation to the potential acid base intake in the food and the degree of oxidation of food material is of importance in fundamental physiological researches and in clinical methods. Besides refer-

ences to be found under "blood" the following are some of the more special references on urine.²

References. Auerbach-Friedenthal (1903), Blatherwick (1914), Bugarszky (1897), Cushny (*book* 1917), Fiske (1920), Fitz-Van Slyke (1917), Foà (1905), Haskins (1919), Hasselbalch (1916), Henderson (1910, 1911, 1914), Henderson-Palmer (1913), Henderson-Spiro (1908), Höber (1902), Höber-Jankowsky (1903), Howe-Hawk (1914), Macleod-Knapp (1918), Nagayma (1920), Nelson-Williams (1916), Newburgh-Palmer-Henderson (1913), Palmer-Henderson (1915), Quagliariello-d'Agostino (1912), Reemelin-Issacs (1916), Rhorer (1901), Ringer (1909, 1910), Skramlik (1911), Stillman-Van Slyke (1917), Talbert (1920), Van Slyke-Palmer (1919).

VINEGAR.

Reference. Brode-Lange (1909).

WATER (sea and fresh). The carbonate equilibrium maintains sea water at a very constant pH which has doubtless varied with the CO₂ tension of the atmosphere in geological ages and which varies somewhat with the temperature, and locally with accretions from rivers and springs and contact with geologic deposits. The wider aspects of the carbonate equilibria involved have been described in Henderson's *Fitness of the Environment*. The charting of the pH values for different regions of the seas has been of aid in oceanographic surveys and in some instances has been of value in the study of plant and animal distribution.

Fresh waters are influenced chiefly by the deposits with which they come in contact. pH determinations in the field are of aid to the geologist in demarking waters of limestone origin (Wherry private communication).

References. Auerbach (1904), Corti-Alvarez (1918), Gaarder (1916-1917), Haas (1916), Henderson (1913), Henderson-Cohn (1916), Loeb (1904), McClendon (1916, 1917), Mayer (1919), Michaelis (1914), Palitzsch (1911, 1915, 1916), Prideaux (1919), Ringer (1908), Rupp, (1909), Shelford (1919), Sørensen-Palitzsch (1910, 1913), Stephanides (1916), Tillmans (1919), Trillat (1916), Walker-Kay (1912).

² See Clark and Lubs (1917) for some examples of the application of the sulfon phthalein indicators to the determination of the pH of urines.

WATER, pure. Ionization of.

References. Kohlrausch-Heydweiller (1894), Lewis, Brighton and Sebastian (1917), Nernst (1894), Ostwald (1893), Wijs (1893).

WINE ACIDITY. Besides influencing the fermentations the pH of wine has been found to correlate in a general way with the acid taste.

References. Dutoit-Dubroux (1910), Paul (1914, 1915, 1916), Quartaroli (1912).

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APPENDIX

TEMPERATURE FACTORS FOR CONCENTRATION CHAINS

$$E = 0.000,198,37 T \log \frac{C_1}{C_2} \text{ (when valence} = 1 \text{)}$$

t (CENTIGRADE)	T (ABSOLUTE)	0.000,198,37 T	LOG 0.000,198,37 T
0	273.09	0.05416	2.73364
5	278.09	0.05515	2.74152
10	283.09	0.05614	2.74927
15	288.09	0.05713	2.75687
16	289.09	0.05733	2.75838
17	290.09	0.05753	2.75988
18	291.09	0.05772	2.76137
19	292.09	0.05792	2.76286
20	293.09	0.05812	2.76435
21	294.09	0.05832	2.76583
22	295.09	0.05852	2.76730
23	296.09	0.05872	2.76877
24	297.09	0.05892	2.77023
25	298.09	0.05911	2.77169
26	299.09	0.05931	2.77315
27	300.09	0.05951	2.77460
28	301.09	0.05971	2.77604
29	302.09	0.05991	2.77749
30	303.09	0.06011	2.77892
31	304.09	0.06031	2.78035
32	305.09	0.06050	2.78178
33	306.09	0.06070	2.78320
34	307.09	0.06090	2.78462
35	308.09	0.06110	2.78603
36	309.09	0.06130	2.78742
37	310.09	0.06150	2.78884
37.5	310.59	0.06159	2.78954
38	311.09	0.06169	2.79024
39	312.09	0.06189	2.79163
40	313.09	0.06209	2.79302
45	318.09	0.06308	2.79990
50	323.09	0.06407	2.80668

CORRECTION OF BAROMETER READING FOR TEMPERATURE

When the mercury in the barometer is at the temperature t subtract the following corrections to obtain the barometric height in terms of mercury at zero degrees centigrade.

t	BAROMETER READINGS IN MILLIMETERS						
	720	730	740	750	760	770	780
17	2.0	2.0	2.1	2.1	2.1	2.1	2.2
18	2.1	2.1	2.2	2.2	2.2	2.3	2.3
19	2.2	2.3	2.3	2.3	2.4	2.4	2.4
20	2.3	2.4	2.4	2.4	2.5	2.5	2.5
21	2.5	2.5	2.5	2.6	2.6	2.6	2.7
22	2.6	2.6	2.7	2.7	2.7	2.8	2.8
23	2.7	2.7	2.8	2.8	2.8	2.9	2.9
24	2.8	2.9	2.9	2.9	3.0	3.0	3.1
25	2.9	3.0	3.0	3.1	3.1	3.1	3.2
26	3.0	3.1	3.1	3.2	3.2	3.3	3.3
27	3.2	3.2	3.3	3.3	3.3	3.4	3.4
28	3.3	3.3	3.4	3.4	3.5	3.5	3.6
29	3.4	3.4	3.5	3.5	3.6	3.6	3.7
30	3.5	3.6	3.6	3.7	3.7	3.8	3.8
31	3.6	3.7	3.7	3.8	3.8	3.9	3.9

BAROMETRIC CORRECTIONS FOR H-ELECTRODE POTENTIALS
(Data for use in plotting correction curves)

$$E_{\text{bar.}} = \frac{0.000,19837 T}{2} \log \frac{760}{x}$$

TEMPER- ATURE	CORRECTED PRESSURE	VAPOR PRESSURE	x	LOG $\frac{760}{x}$	E _{bar.}
°C.	mm.	mm.			millivolts
18	780	15.5	764.5	-0.00256	-0.07
	760		744.5	0.00895	0.26
	740		724.5	0.02078	0.60
20	780	17.5	762.5	-0.00143	-0.04
	760		742.5	0.01012	0.29
	740		722.5	0.02198	0.64
25	780	23.8	756.2	0.00218	0.06
	760		736.2	0.01382	0.41
	740		716.2	0.02578	0.76
30	780	31.8	748.2	0.00680	0.20
	760		728.2	0.01856	0.56
	740		708.2	0.03066	0.92
35	780	42.2	737.8	0.01288	0.39
	760		717.8	0.02481	0.76
	740		697.8	0.03708	1.13
40	780	55.3	724.8	0.02060	0.64
	760		704.8	0.03275	1.02
	740		684.7	0.04525	1.41

$$\frac{E. M. F. + E_{\text{bar.}} - E_{\text{cal.}}}{0.000,19837 T} = \text{pH}$$

STANDARD* VALUES FOR CALOMEL ELECTRODES
(Referred to the normal hydrogen electrode)

TEMPERATURE	CONCENTRATION OF KCl		
	M/10	M/1	Saturated (approximate potential)
°C.			
18	0.3380	0.2864	0.2506
20	0.3379	0.2860	0.2492
25	0.3376	0.2848	0.2464
30	0.3373	0.2837	0.2437
40	0.3360		

* See page 203.

VALUES OF $\text{LOG } \frac{\alpha}{1 - \alpha}$ FOR USE IN CONSTRUCTING DISSOCIATION CURVES

DISSOCIATION	α	$\text{LOG } \frac{\alpha}{(1 - \alpha)}$
<i>per cent</i>		
1	0.01	-1.995
2	0.02	-1.690
3	0.03	-1.510
5	0.05	-1.279
10	0.1	-0.954
20	0.2	-0.602
30	0.3	-0.367
40	0.4	-0.176
50	0.5	0.000
60	0.6	0.176
70	0.7	0.367
80	0.8	0.602
90	0.9	0.954
95	0.95	1.279
97	0.97	1.510
98	0.98	1.690
99	0.99	1.995

TABLE SHOWING RELATION OF $[H^+]$ TO pH(On the assumption that $pH = \log \frac{1}{[H^+]}$, see Chapter XVII)

pH	$[H^+]$
x.00	1.00×10^{-x}
x.05	0.89×10^{-x}
x.10	0.79×10^{-x}
x.15	0.71×10^{-x}
x.20	0.63×10^{-x}
x.25	0.56×10^{-x}
x.30	0.50×10^{-x}
x.35	0.45×10^{-x}
x.40	0.40×10^{-x}
x.45	0.36×10^{-x}
x.50	0.32×10^{-x}
x.55	0.28×10^{-x}
x.60	0.25×10^{-x}
x.65	0.22×10^{-x}
x.70	0.20×10^{-x}
x.75	0.18×10^{-x}
x.80	0.16×10^{-x}
x.85	0.14×10^{-x}
x.90	0.13×10^{-x}
x.95	0.11×10^{-x}
x + 1.00	0.10×10^{-x}

Example: $pH = 7.00$; $[H^+] = 1 \times 10^{-7}$ $pH = 7.60$; $[H^+] = 0.25 \times 10^{-7}$ or 2.5×10^{-8}

Compare Symes (1916).

IONIZATION CONSTANTS

The following list of ionization constants was compiled from Scudder's *Conductivity and Ionization Constants of Organic Compounds*, 1914.

Acetic acid.....	K_a	1.8×10^{-5}
Alloxan.....	K_a	2.3×10^{-7}
Amino acetic acid (glycine).....	K_a	1.8×10^{-10}
	K_b	2.8×10^{-12}
α -alanine.....	K_a	2.0×10^{-10}
	K_b	3.0×10^{-12}
Ammonium hydroxid.....	K_b	1.8×10^{-5}
Aspartic acid.....	K_a	1.4×10^{-4}
	K_b	1.2×10^{-12}
Asparagine.....	K_a	1.4×10^{-9}
	K_b	1.5×10^{-12}
Butyric acid.....	K_a	1.6×10^{-5}
Cacodylic acid.....	K_a	7.5×10^{-7}
	K_b	3.8×10^{-13}
Citric acid.....	K_{a1}	8.2×10^{-4}
	K_{a2}	3.2×10^{-5}
	K_{a3}	7.0×10^{-7}
Formic acid.....	K_a	2.1×10^{-4}
Fructose.....	K_a	8.8×10^{-13}
Glutamic acid.....	K_a	4.1×10^{-5}
Glucose.....	K_a	5.9×10^{-13}
Hippuric acid.....	K_a	2.2×10^{-4}
	K_b	2.2×10^{-9}
Histidine.....	K_{b1}	5.7×10^{-9}
	K_{b2}	5.0×10^{-13}
Lactic acid.....	K_a	1.4×10^{-4}
Lysine.....	K_a	1.0×10^{-11}
	K_b	1.0×10^{-7}
Mucic acid.....	K_a	6.3×10^{-4}
Nitrous acid.....	K_a	6.0×10^{-4}
Oxalic acid.....	K_{a1}	1.0×10^{-1}
	K_{a2}	4.1×10^{-5}
O-phthalic acid.....	K_{a1}	1.2×10^{-3}
	K_{a2}	3.9×10^{-6}
Succinic acid.....	K_{a1}	6.8×10^{-5}
	K_{a2}	2.7×10^{-6}
d-Tartaric acid.....	K_{a1}	9.7×10^{-4}
	K_{a2}	4.5×10^{-5}
Tyrosine.....	K_{a1}	4.0×10^{-9}
	K_{a2}	4.0×10^{-10}
	K_b	2.6×10^{-12}
Urea.....	K_b	1.5×10^{-14}
Uric acid.....	K_a	1.5×10^{-6}

REPRESENTATIVE POTENTIOMETER EQUIPMENT

I. The author's equipment

Leeds and Northrup type K potentiometer. Leeds and Northrup type R galvanometer, sensitivity 1973 megohms, galvanometer resistance 510 ohms, critical damping resistance 10,000 ohms, period 5.4 seconds. Student's decade resistance box for critical damping resistance, Julius suspension for galvanometer. Telescope and scale with adjustments. Two Weston commercial standard cells. Two normal Weston standard cells. Two two-volt, 60 ampere hours storage batteries. Portable volt meter, range four volts, for testing storage cells. Switches for switch board.

II. Resistance box system

Two decade resistance boxes each furnishing a total resistance of 9999 ohms. Resistance unit of exactly 182 ohms if standard Weston cell has E. M. F. of 1.0181 volts; otherwise resistance unit which added to 9999 ohms will give 10,000 times in ohms the numerical value of the voltage of the Weston cell to be used. Variable rheostat for adjusting battery current to give 0.0001 ampere. Two volt storage cell. Switches. Galvanometer.

III. Kohlrausch slide wire system

(Not recommended.) If the slide wire has a resistance of 7 ohms provide regulating rheostat of 6 to 8 ohms and a resistance unit or box furnishing 0.128 ohms. The extra resistance unit is placed in series with the slide wire to furnish the resistance required for throwing in a Weston cell should the wire be calibrated for a total potential difference of one volt. Two volt storage battery. Switches. Portable galvanometer or capillary electrometer.

IV. Millivoltmeter system

For rough measurements, see fig. 25. Millivoltmeter, range 1 volt, scale divisions 0.01 volt. Slide wire resistance. (This slide wire need not be calibrated but the most convenient form will be found in the drum wound Kohlrausch slide wires used in bridge measurements.) Dry cell or storage cell. Regulating rheostat of range suitable for adjusting current from battery to furnish about one volt difference of potential between ends of slide wire. Switches. Capillary electrometer or portable galvanometer (with 1000 ohms coil resistance preferable.)

Note. In place of the millivoltmeter a milliammeter may be used as follows. Provide a fixed and accurately known resistance at the terminals of which the terminals of the measured system may be connected. Place in series with the fixed resistance a regulating rheostat. Adjust fixed resistance and rheostat to make use of the full range of the milliammeter and to obtain balance regulate current flowing through the fixed resistance. Calibrate ammeter scale in volts or adjust system so that scale divisions correspond to fractions of a volt.

LOGARITHMS OF NUMBERS

NATURAL NUMBERS											PROPORTIONAL PARTS									
	0	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	
10	0000	0043	0086	0128	0170	0212	0253	0294	0334	0374	4	8	12	17	21	25	29	33	37	
11	0414	0453	0492	0531	0569	0607	0645	0682	0719	0755	4	8	11	15	19	23	26	30	34	
12	0792	0828	0864	0899	0934	0969	1004	1038	1072	1106	3	7	10	14	17	21	24	28	31	
13	1139	1173	1206	1239	1271	1303	1335	1367	1399	1430	3	6	10	13	16	19	23	26	29	
14	1461	1492	1523	1553	1584	1614	1644	1673	1703	1732	3	6	9	12	15	18	21	24	27	
15	1761	1790	1818	1847	1875	1903	1931	1959	1987	2014	3	6	8	11	14	17	20	22	25	
16	2041	2068	2095	2122	2148	2175	2201	2227	2253	2279	3	5	8	11	13	16	18	21	24	
17	2304	2330	2355	2380	2405	2430	2455	2480	2504	2529	2	5	7	10	12	15	17	20	22	
18	2553	2577	2601	2625	2648	2672	2695	2718	2742	2765	2	5	7	9	12	14	16	19	21	
19	2788	2810	2833	2856	2878	2900	2923	2945	2967	2989	2	4	7	9	11	13	16	18	20	
20	3010	3032	3054	3075	3096	3118	3139	3160	3181	3201	2	4	6	8	11	13	15	17	19	
21	3222	3243	3263	3284	3304	3324	3345	3365	3385	3404	2	4	6	8	10	12	14	16	18	
22	3424	3444	3464	3483	3502	3522	3541	3560	3579	3598	2	4	6	8	10	12	14	15	17	
23	3617	3636	3655	3674	3692	3711	3729	3747	3766	3784	2	4	6	7	9	11	13	15	17	
24	3802	3820	3838	3856	3874	3892	3909	3927	3945	3962	2	4	5	7	9	11	12	14	16	
25	3979	3997	4014	4031	4048	4065	4082	4099	4116	4133	2	3	5	7	9	10	12	14	15	
26	4150	4166	4183	4200	4216	4232	4249	4265	4281	4298	2	3	5	7	8	10	11	13	15	
27	4314	4330	4346	4362	4378	4393	4409	4425	4440	4456	2	3	5	6	8	9	11	13	14	
28	4472	4487	4502	4518	4533	4548	4564	4579	4594	4609	2	3	5	6	8	9	11	12	14	
29	4624	4639	4654	4669	4683	4698	4713	4728	4742	4757	1	3	4	6	7	9	10	12	13	
30	4771	4786	4800	4814	4829	4843	4857	4871	4886	4900	1	3	4	6	7	9	10	11	13	
31	4914	4928	4942	4955	4969	4983	4997	5011	5024	5038	1	3	4	6	7	8	10	11	12	
32	5052	5065	5079	5092	5105	5119	5132	5145	5159	5172	1	3	4	5	7	8	9	11	12	
33	5185	5198	5211	5224	5237	5250	5263	5276	5289	5302	1	3	4	5	6	8	9	10	12	
34	5315	5328	5340	5353	5366	5378	5391	5403	5416	5428	1	3	4	5	6	8	9	10	11	
35	5441	5453	5465	5478	5490	5502	5514	5527	5539	5551	1	2	4	5	6	7	9	10	11	
36	5563	5575	5587	5599	5611	5623	5635	5647	5658	5670	1	2	4	5	6	7	8	10	11	
37	5682	5694	5705	5717	5729	5740	5752	5763	5775	5786	1	2	3	5	6	7	8	9	10	
38	5798	5809	5821	5832	5843	5855	5866	5877	5888	5899	1	2	3	5	6	7	8	9	10	
39	5911	5922	5933	5944	5955	5966	5977	5988	5999	6010	1	2	3	4	5	7	8	9	10	
40	6021	6031	6042	6053	6064	6075	6085	6096	6107	6117	1	2	3	4	5	6	8	9	10	
41	6128	6138	6149	6160	6170	6180	6191	6201	6212	6222	1	2	3	4	5	6	7	8	9	
42	6232	6243	6253	6263	6274	6284	6294	6304	6314	6325	1	2	3	4	5	6	7	8	9	
43	6335	6345	6355	6365	6375	6385	6395	6405	6415	6425	1	2	3	4	5	6	7	8	9	
44	6435	6444	6454	6464	6474	6484	6493	6503	6513	6522	1	2	3	4	5	6	7	8	9	
45	6532	6542	6551	6561	6571	6580	6590	6599	6609	6618	1	2	3	4	5	6	7	8	9	
46	6628	6637	6646	6656	6665	6675	6684	6693	6702	6712	1	2	3	4	5	6	7	7	8	
47	6721	6730	6739	6749	6758	6767	6776	6785	6794	6803	1	2	3	4	5	5	6	7	8	
48	6812	6821	6830	6839	6848	6857	6866	6875	6884	6893	1	2	3	4	4	5	6	7	8	
49	6902	6911	6920	6928	6937	6946	6955	6964	6972	6981	1	2	3	4	4	5	6	7	8	
50	6990	6998	7007	7016	7024	7033	7042	7050	7059	7067	1	2	3	3	4	5	6	7	8	
51	7076	7084	7093	7101	7110	7118	7126	7135	7143	7152	1	2	3	3	4	5	6	7	8	
52	7160	7168	7177	7185	7193	7202	7210	7218	7226	7235	1	2	2	3	4	5	6	7	7	
53	7243	7251	7259	7267	7275	7284	7292	7300	7308	7316	1	2	2	3	4	5	6	6	7	
54	7324	7332	7340	7348	7356	7364	7372	7380	7388	7396	1	2	2	3	4	5	6	6	7	

NATURAL NUMBERS											PROPORTIONAL PARTS								
	0	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
55	7404	7412	7419	7427	7435	7443	7451	7459	7466	7474	1	2	2	3	4	5	5	6	7
56	7482	7490	7497	7505	7513	7520	7528	7536	7543	7551	1	2	2	3	4	5	5	6	7
57	7559	7566	7574	7582	7589	7597	7604	7612	7619	7627	1	2	2	3	4	5	5	6	7
58	7634	7642	7649	7657	7664	7672	7679	7686	7694	7701	1	1	2	3	4	4	5	6	7
59	7709	7716	7723	7731	7738	7745	7752	7760	7767	7774	1	1	2	3	4	4	5	6	7
60	7782	7789	7796	7803	7810	7818	7825	7832	7839	7846	1	1	2	3	4	4	5	6	6
61	7853	7860	7868	7875	7882	7889	7896	7903	7910	7917	1	1	2	3	4	4	5	6	6
62	7924	7931	7938	7945	7952	7959	7966	7973	7980	7987	1	1	2	3	3	4	5	6	6
63	7993	8000	8007	8014	8021	8028	8035	8041	8048	8055	1	1	2	3	3	4	5	5	6
64	8062	8069	8075	8082	8089	8096	8102	8109	8116	8122	1	1	2	3	3	4	5	5	6
65	8129	8136	8142	8149	8156	8162	8169	8176	8182	8189	1	1	2	3	3	4	5	5	6
66	8195	8202	8209	8215	8222	8228	8235	8241	8248	8254	1	1	2	3	3	4	5	5	6
67	8261	8267	8274	8280	8287	8293	8299	8306	8312	8319	1	1	2	3	3	4	5	5	6
68	8325	8331	8338	8344	8351	8357	8363	8370	8376	8382	1	1	2	3	3	4	4	5	6
69	8388	8395	8401	8407	8414	8420	8426	8432	8439	8445	1	1	2	2	3	4	4	5	6
70	8451	8457	8463	8470	8476	8482	8488	8494	8500	8506	1	1	2	2	3	4	4	5	6
71	8513	8519	8525	8531	8537	8543	8549	8555	8561	8567	1	1	2	2	3	4	4	5	5
72	8573	8579	8585	8591	8597	8603	8609	8615	8621	8627	1	1	2	2	3	4	4	5	5
73	8633	8639	8645	8651	8657	8663	8669	8675	8681	8686	1	1	2	2	3	4	4	5	5
74	8692	8698	8704	8710	8716	8722	8727	8733	8739	8745	1	1	2	2	3	4	4	5	5
75	8751	8756	8762	8768	8774	8779	8785	8791	8797	8802	1	1	2	2	3	3	4	5	5
76	8808	8814	8820	8825	8831	8837	8842	8848	8854	8859	1	1	2	2	3	3	4	5	5
77	8865	8871	8876	8882	8887	8893	8899	8904	8910	8915	1	1	2	2	3	3	4	4	5
78	8921	8927	8932	8938	8943	8949	8954	8960	8965	8971	1	1	2	2	3	3	4	4	5
79	8976	8982	8987	8993	8998	9004	9009	9015	9020	9025	1	1	2	2	3	3	4	4	5
80	9031	9036	9042	9047	9053	9058	9063	9069	9074	9079	1	1	2	2	3	3	4	4	5
81	9085	9090	9096	9101	9106	9112	9117	9122	9128	9133	1	1	2	2	3	3	4	4	5
82	9138	9143	9149	9154	9159	9165	9170	9175	9180	9186	1	1	2	2	3	3	4	4	5
83	9191	9196	9201	9206	9212	9217	9222	9227	9232	9238	1	1	2	2	3	3	4	4	5
84	9243	9248	9253	9258	9263	9269	9274	9279	9284	9289	1	1	2	2	3	3	4	4	5
85	9294	9299	9304	9309	9315	9320	9325	9330	9335	9340	1	1	2	2	3	3	4	4	5
86	9345	9350	9355	9360	9365	9370	9375	9380	9385	9390	1	1	2	2	3	3	4	4	5
87	9395	9400	9405	9410	9415	9420	9425	9430	9435	9440	0	1	1	2	2	3	3	4	4
88	9445	9450	9455	9460	9465	9469	9474	9479	9484	9489	0	1	1	2	2	3	3	4	4
89	9494	9499	9504	9509	9513	9518	9523	9528	9533	9538	0	1	1	2	2	3	3	4	4
90	9542	9547	9552	9557	9562	9566	9571	9576	9581	9586	0	1	1	2	2	3	3	4	4
91	9590	9595	9600	9605	9609	9614	9619	9624	9628	9633	0	1	1	2	2	3	3	4	4
92	9638	9643	9647	9652	9657	9661	9666	9671	9675	9680	0	1	1	2	2	3	3	4	4
93	9685	9689	9694	9699	9703	9708	9713	9717	9722	9727	0	1	1	2	2	3	3	4	4
94	9731	9736	9741	9745	9750	9754	9759	9763	9768	9773	0	1	1	2	2	3	3	4	4
95	9777	9782	9786	9791	9795	9800	9805	9809	9814	9818	0	1	1	2	2	3	3	4	4
96	9823	9827	9832	9836	9841	9845	9850	9854	9859	9863	0	1	1	2	2	3	3	4	4
97	9868	9872	9877	9881	9886	9890	9894	9899	9903	9908	0	1	1	2	2	3	3	4	4
98	9912	9917	9921	9926	9930	9934	9939	9943	9948	9952	0	1	1	2	2	3	3	4	4
99	9956	9961	9965	9969	9974	9978	9983	9987	9991	9996	0	1	1	2	2	3	3	4	4

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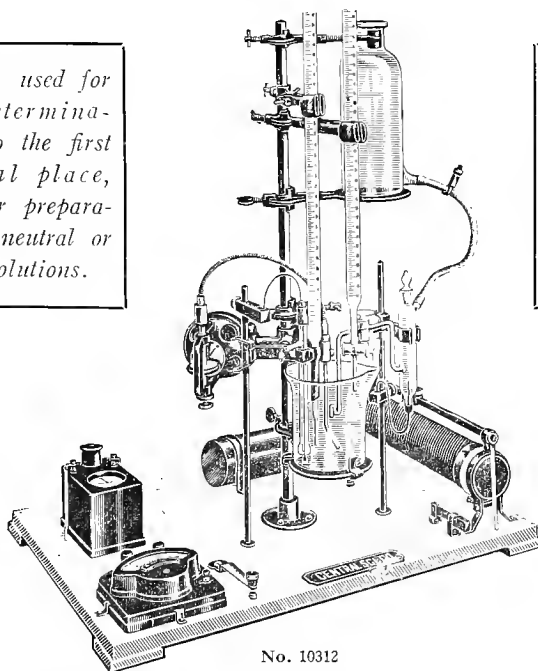
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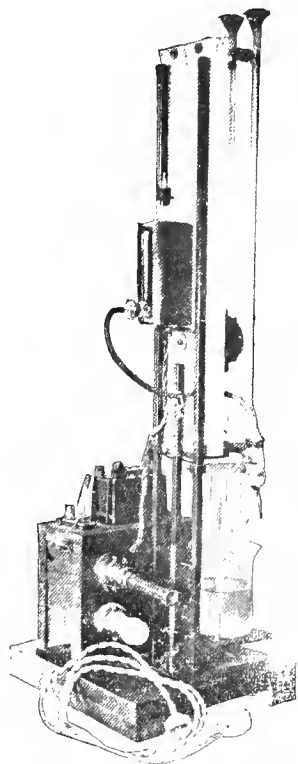
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Brom-Thymol Blue	Dibromthymolsulphonephthalein	6.0—7.6
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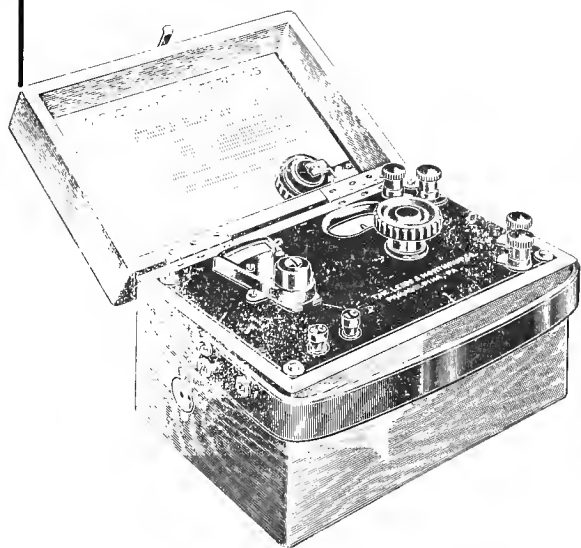
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per cent solution	
Brom phenol blue (B.P.B.).....	0.5 cc. 0.04
per cent solution	
Methyl red (M.R.).....	0.3 cc. 0.02
per cent solution	
Brom cresol purple (B.C.P.).....	0.5 cc. 0.04
per cent solution	
Brom thymol blue (B.T.B.).....	0.5 cc. 0.04
per cent solution	
Phenol red (P.R.).....	0.5 cc. 0.02
per cent solution	
Cresol red (C.R.).....	0.5 cc. 0.02
per cent solution	
Thymol blue (T.B.).....	0.5 cc. 0.04
per cent solution	

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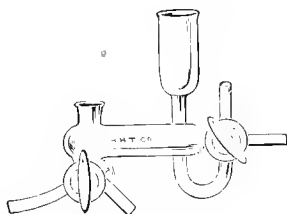
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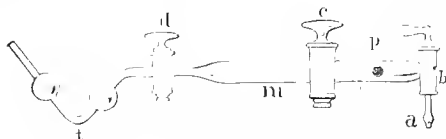
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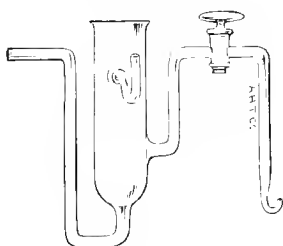
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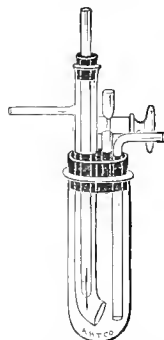
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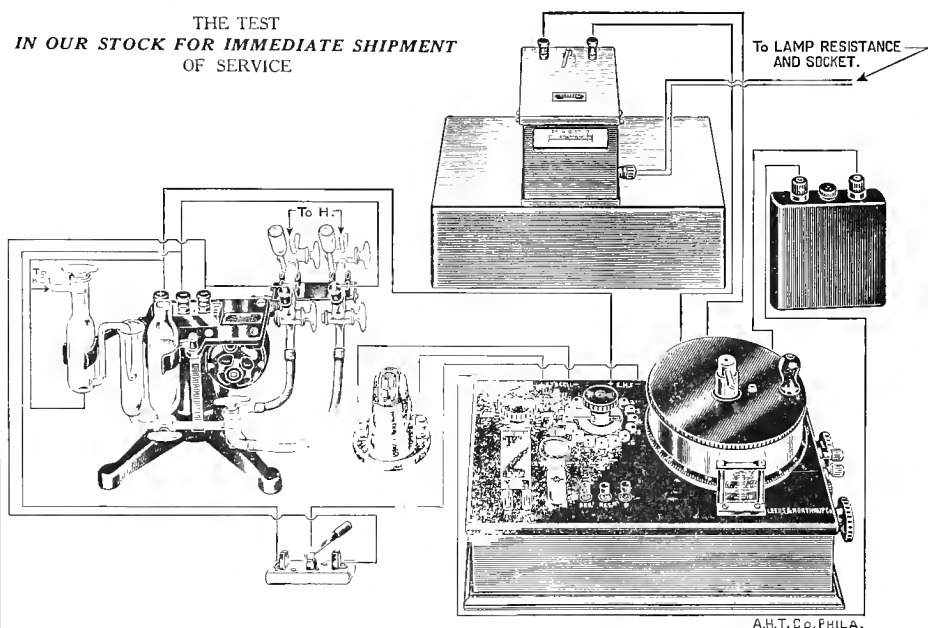
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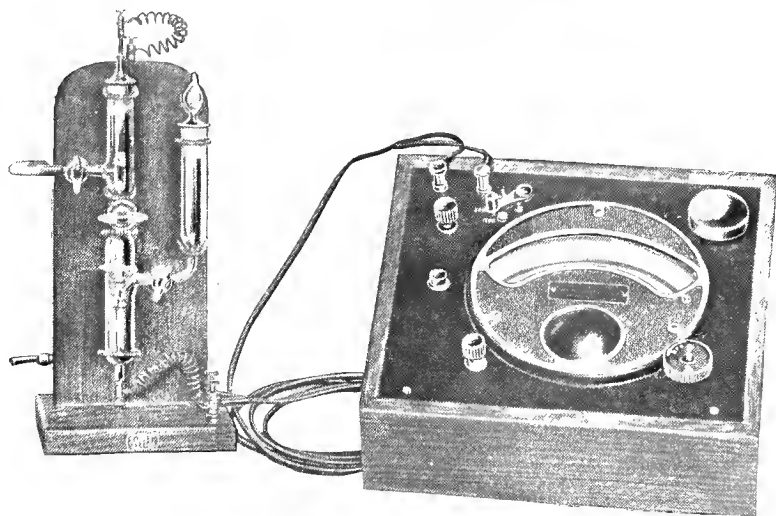
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